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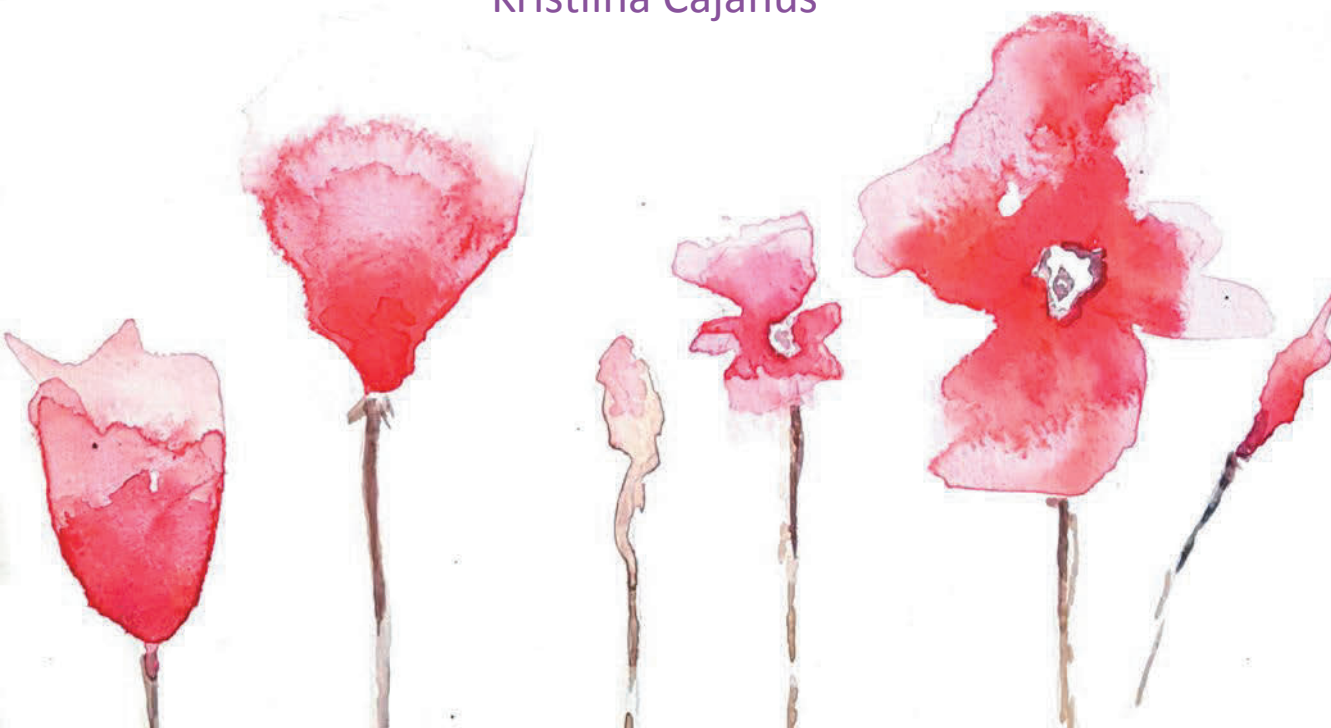
Anaesthesiology,
Intensive Care and Pain Medicine
Faculty of Medicine
Doctoral Programme in Clinical Research
University of Helsinki

Factors Affecting Pain Intensity and Postoperative Oxycodone Requirement

Clinical and Genetic Perspectives

Kristiina Cajanus



Division of Pain Medicine,
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Faculty of Medicine
Doctoral Programme in Clinical Research

Academic Dissertation

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In a world full of Kardashians, be a Curie.

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications and some unpublished data.

I. **Cajanus K, Neuvonen M, Koskela O, Kaunisto MA, Neuvonen PJ, Niemi M, Kalso E. Analgesic plasma concentrations of oxycodone after surgery for breast cancer – which factors matter? Clinical Pharmacology and Therapeutics 2018, 103 (4): 653-662**

II. **Cajanus K, Kaunisto MA, Tallgren M, Jokela R, Kalso E. How Much Oxycodone Is Needed for Adequate Analgesia After Breast Cancer Surgery: Effect of the *OPRM1* rs1799971 Polymorphism. The Journal of Pain 2014, 15 (12): 1248-1256.**

III. **Cajanus K, Holmstrom EJ, Wessman M, Anttila V, Kaunisto MA, Kalso E. Effect of endocannabinoid degradation on pain: role of *FAAH* polymorphisms in experimental and postoperative pain in women treated for breast cancer. Pain 2016; 157 (2): 361-369.**

LIST OF ABBREVIATIONS

2-AG	2-arachidonoyl glycerol
3'UTR	Three prime untranslated region
5'UTR	Five prime untranslated region
AC	Axillary clearance
AEA	N-arachidonoyl ethanolamide, Anandamide
AUC	Area under curve
BBB	Blood-brain barrier
BCS	Breast conserving surgery
BDI	Beck Depression Inventory
BMI	Body mass index
c-AMP	Cyclic adenosine monophosphate
CAR	Constitutive androstane receptor
CB_1	Cannabinoid receptor type 1
CB_2	Cannabinoid receptor type 2
CNS	Central nervous system
COX-2	Cyclo-oxygenase 2
CSF	Cerebrospinal fluid
CYP	Cytochrome P-450
DNA	Deoxyribonucleic acid
EM	Extensive metabolizer
FAAH	Fatty acid amide hydrolase
G protein	Guanine nucleotide-binding protein
GABA	Gamma-aminobutyric acid
GWAS	Genome wide association study
HIV	Human immunodefiency virus
HWE	Hardy-Weinberg equation
IAE	Idiopathic absence epilepsy
IGE	Idiopathic generalized epilepsy
IM	Intermediate metabolizer
iv.	Intravenous
mRNA	messenger RNA
NRS	Numerical rating scale
NSAID	Non-steroidal anti-inflammatory drug
PACU	Postoperative care unit
PAG	Periaqueductal grey
PCA	Patient controlled analgesia device
PM	Poor metabolizer
PPAR	Peroxisome proliferator activated receptor
PTSD	Post-traumatic stress disorder
PXR	Pregnane X receptor

RNA	Ribonucleic acid
RVM	Rostroventromedial medulla
SD	Standard deviation
SNB	Sentinel node biopsy
SNP	Single nucleotide polymorphism
STAI	State-Trait Anxiety Inventory
TRPA1	Transient receptor potential cation channel, subfamily A, member 1
TRPM8	Transient receptor potential cation channel, subfamily M, member 8
TRPV1	Transient receptor potential cation channel, subfamily V, member 1
TRPV2	Transient receptor potential cation channel, subfamily V, member 2
TRPV3	Transient receptor potential cation channel, subfamily V, member 3
TRPV4	Transient receptor potential cation channel, subfamily V, member 4
UM	Ultra-rapid metabolizer
VAS	Visual analog scale

ABSTRACT

Breast cancer is one of the most common cancers in the world. The advancement in diagnostic tools and treatment options have majorly improved the prognosis of the disease. Nevertheless, every year thousands of women receive this diagnosis and undergo breast cancer surgery in hope of survival.

Modern day surgery requires efficient analgesia to protect the body from the stress caused by severe pain. Therefore, analgesics are used both intra- and post-operatively. Opioids are the gold standard analgesic for moderate or severe post-operative pain and oxycodone has been the opioid most used for post-operative pain in Finland for decades. Due to the serious adverse effects, all opioids must be titrated to analgesic doses gradually, which prolongs the time it takes to reach satisfactory analgesia and predisposes patient to suffering and to the harmful physical effects of pain. The purpose of this thesis was to identify whether oxycodone has an analgesic plasma concentration, as well as which factors affect the post-operative pain intensity and oxycodone requirements after breast cancer surgery. The identification of these factors helps clinicians recognize patients at risk of severe post-operative pain and in need of higher doses of oxycodone to reach satisfactory analgesia.

The study cohort consisted of 1,000 women about to undergo breast cancer surgery due to unilateral non-metastasised breast cancer. Before the surgery, each patient`s demographic data, medical history, pain, depressive symptoms and anxiety state were recorded along with heat (48°C) and cold (4°C) pain sensitivity. Anaesthesia protocol was standardized. After the surgery, all patients were titrated to satisfactory analgesia with intravenous oxycodone. The post-operative pain intensities and oxycodone requirements were recorded from the first 20 post-operative hours.

Post-operative pain intensity, requirement of oxycodone and analgesic oxycodone concentrations varied significantly between patients. Factors associated with higher post-operative motion pain were young age, axillary clearance (vs. sentinel node biopsy), higher preoperative cold pain intensity and cold pain tolerance. Together these factors explained 8.5% of the variation in the motion pain intensities. Post-operative motion pain intensity was the major predictor of post-operative oxycodone requirement, together with BMI, age, axillary clearance, OPRM1 rs1799971 genotype and preoperative breast pain. Together these factors explained 28% of the total variation in oxycodone requirement. Only post-operative motion pain intensity and axillary clearance were associated with analgesic oxycodone plasma concentrations, which together explained almost 17% of the total variation.

CYP2D6 genotype did not associate with the analgesic oxycodone requirement or concentration but did affect the oxymorphone and noroxymorphone plasma concentrations.

The results presented in this study could help identify patients at risk of higher pain intensities and oxycodone requirements after breast cancer surgery.

TIIVISTELMÄ

Rintasyöpä on yksi mailman yleisimmistä syövistä. Diagnostiikan ja hoitokeinojen kehittyminen on merkittävästi parantunut taudin ennustetta. Kuitenkin joka vuosi tuhannet naiset saavat rintasyöpädiagnoosin ja läpikäyvät rintasyöpäleikkauksen toivoen parantumista.

Nykyaikainen kirurginen hoito vaatii tehokasta kivunlievitystä, sillä voimakas kipu altistaa elimistön merkittävälle rasitukselle. Kivun lievittämiseksi leikkauksen aikana ja sen jälkeen potilaalle annostellaan kipulääkkeitä, joista opioidit ovat käytetyin kipulääkeryhmä keskivaikean ja vaikean leikkauksen jälkeisen kivun hoidossa. Opioidihoitoon liittyy kuitenkin vakavia haittavaikutuksia, kuten pahoinvointi ja hengityslama, joiden vuoksi kaikki opioidit joudutaan titraamaan kipua lievittävään annokseen asteittain. Tämä pitkittää riittävän kivunlievityksen saavuttamiseen kuluvaa aikaa ja altistaa potilaan kivun aiheuttamalle kärsimykselle ja elimistön kuormitukselle. Oksikodoni on ollut vuosikausia Suomen käytetyin opioidi leikkauksen jälkeisen kivun hoidossa. Tämän väitöskirjan tarkoitus oli tutkia, onko oksikodonille määritettävissä analgeettista pitoisuutta sekä määrittää leikkauksen jälkeisen kivun voimakkuuteen ja oksikodonin tarpeeseen vaikuttavia tekijöitä. Näiden tekijöiden tunnistaminen helpottaisi riskipotilaiden tunnistamista sekä voimakkaan leikkauksen jälkeisen kivun että suuren oksikodonin tarpeen suhteen. Näin hoitohenkilökunta voisi jo ennalta varautua haastavaan postoperatiivisen kivun lievitykseen.

Tutkimuspopulaatio koostui 1000 naisesta, jotka olivat menossa rintaleikkaukseen diagnosoidun rintasyövän vuoksi. Potilailla oli vain toisen rinnan tauti eikä heillä ollut havaittu etäpesäkkeitä. Ennen leikkausta potilaat täyttivät kyselyt demografisista tiedoistaan, sairaushistoriastaan, kipuoireistaan sekä masennus- ja ahdistustasostaan. Lisäksi potilaiden herkkyys kokeelliseessa kuuma- ja kylmä-kipukokeessa sekä kylmäkivun sieto testattiin. Tutkimuspotilaiden anestesia oli vakioitu. Leikkauksen jälkeen kaikkille potilaille annosteltiin suonensisäistä oksikodonia kunnes he ilmaisivat kivunlievityksen olevan riittävä. Leikkauksen jälkeinen kivun voimakkuus ja oksikodonin käyttö kirjattiin ensimmäisen 20 leikkauksen jälkeisen tunnin ajalta.

Hyvään kivunlievitykseen tarvittavat plasman oksikodonipitoisuuden vaihtelivat huomattavasti potilaiden kesken. Leikkauksen jälkeiseen liikekivun voimakkuuteen liittyvät tekijät olivat nuori ikä, kainalon imusolmukkeiden poisto (vs. vartijaimusolmukebiopsia), suurempi herkkyys kylmäkivulle ja vähäisempi kylmäkivun sieto. Nämä tekijät selittivät yhteensä 8,5 % leikkauksen jälkeisen liikekivun voimakkuudesta. Lekkauksen jälkeinen liikekivun voimakkuus puolestaan liittyi vahvasti leikkauksen jälkeiseen oksikodonin kulutukseen samoin kuin painoindeksi, ikä, kainalon imusolmukkeiden poisto, *OPRM1* genotyyppi ja leikkausta edeltänyt kipu rinnassa. Nämä

tekijät selittivät jopa 28 % oksikodonin tarpeen kokonaisvaihtelusta. Vain leikkauksen jälkeinen liikekivun voimakkuus ja kainalon imusolmukkeiden poisto liittyivät oksikodonin analgeettiseen plasmapitoisuuteen. Yhdessä ne selittivät 17 % tämän vaihtelusta. *CYP2D6* genotyyppi ei vaikuttanut leikkauksen jälkeiseen oksikodonitarpeeseen tai analgeettiseen plasmapitoisuuteen, mutta se vaikutti leikkauksen jälkeisiin oksikodonin metaboliittien oksimorfonin ja noroksimorfonin plasman pitoisuuksiin.

Väitöskirjan tulokset auttavat tunnistamaan rintasyöpäleikkauksen läpikäyneitä potilaita, joilla on kohonnut riski voimakkaalle leikkauksen jälkeiselle kivulle sekä suurelle oksikodonin tarpeelle.

1. INTRODUCTION

Opioids, including oxycodone, are widely used for acute post-operative pain. However, their administration is often complicated by the extensive variations in therapeutic range of these drugs. Due to highly individual dosage requirements, opioids must be slowly titrated to analgesic plasma concentrations thus delaying satisfactory analgesia. In order to hasten the achievement of satisfactory analgesia it is critical to have tools to predict the patient's oxycodone requirement. Many studies have found clinical factors that act as such predictors, for example preoperative anxiety, age and preoperative chronic pain. (Ip et al., 2009). Different types of surgery predispose patients to different post-operative pain intensities and opioid requirements according to the invasiveness of surgery and the tissues operated. (Ip et al., 2009; Chiang et al., 2016).

Most studies have used opioid consumption as a proxy for post-operative pain and its relief, although many factors interfere with the administered dose and the final central nervous system (CNS) concentration. One of these factors is the metabolism of oxycodone by the cytochrome P450 enzyme family. The major metabolic routes of oxycodone involve CYP3A4/5, which metabolises oxycodone to noroxycodone and CYP2D6, which in turn produces oxymorphone. The activity of these two enzymes could significantly alter the amount of oxycodone reaching the CNS and therefore the effect oxycodone analgesia. The activity of CYP2D6 is greatly affected by genetic variants, for example, in some individuals, the enzyme is totally ineffective whereas in others the enzyme activity is greatly increased. CYP3A4/5 is the major metaboliser of many clinically used drugs and as a result, the function of the enzymatic pathway is susceptible to both interactions with other drugs as well as genetic variation.

The desired pharmacodynamic effects of opioids take place in opioid receptors in the CNS. Thus, to achieve the most accurate depiction of opioid requirements, the patient's oxycodone concentration should be measured directly from the CSF. The invasiveness of the CSF collection and the possible side effects (e.g. headache) make the study of CSF concentrations both costly and hard to execute. However, even CSF concentrations cannot fully reflect the concentrations at the site of action, since opioid receptors are widely scattered around the CNS and peripheral nervous system. So far, to the best of the author's knowledge, only one previous study has measured the analgesic cerebrospinal fluid oxycodone concentrations (Kokki et al., 2014). Nonetheless, a few studies have investigated the analgesic plasma oxycodone concentrations and the factors predicting analgesic oxycodone plasma concentrations. Of which, three studies have aimed to find a therapeutic range of plasma oxycodone in laparoscopic cholecystectomy (Piirainen et al., 2015; Kokki et al., 2012b; Kokki et al., 2012a).

The increasing focus regarding individual differences in experiencing pain is based on genetics. In the studies of this thesis, we have focused on the genes potentially affecting experimental and postoperative pain intensity as well as oxycodone analgesia: *OPRM1*, *FAAH, CYP2D6* and *CYP3A4/5*.

OPRM1 codes for the mu opioid receptor that is partly responsible for the CNS effects (analgesia, drowsiness etc.) of opioids together with kappa and delta receptors. The opioid system is also highly involved in the regulation of anxiety (Colasanti et al., 2011) and blocking the opioid receptors by naloxone has been shown to increase the self-rated anxiety in a dose dependent manner (Pickar et al, 1982). In the *OPRM1* gene, a single nucleotide polymorphism (SNP) is of special interest since it leads to an amino acid substitution from aspartate to asparagine, therefore changing the N-glycosylation of the mu receptor protein (Huang et al., 2012). The effects of this rs1799971 polymorphism have been studied with regard to both exo- and endogenous opioids. The amino acid substitution alters the binding potential of the receptor for endogenous opioid β-endorphin and possibly to other opioids as well (Bond et al., 1998). In addition, it has also been shown to decrease the receptor's stability in mice (Huang et al., 2012). Other studies that have focussed on the effects of rs1799971 polymorphism on opioid analgesia have mainly used morphine as their drug of choice, although so far the results have been inconclusive, whereas studies devoted to rs1799971 and oxycodone analgesia are rare.

FAAH codes for fatty acid amide hydrolase, a protein degrading endogenous cannabinoids in both the CNS and periphery. The enzyme can degrade many substrates including the main endocannabinoid anandamide (AEA), which is released to the synaptic cleft, where it binds to CB_1 and CB_2 receptors and is then internalized to the neuron. Intracellular FAAH inactivates AEA by breaking it into arachidonic acid and ethanolamine.

Since FAAH activity affects the AEA concentration and thus the endocannabinoid-mediated analgesia, it is an interesting object to study from both a pharmacogenetic and pharmacological aspects. Endocannabinoids have also been shown to participate in the regulation of anxiety in animal and human models (Dincheva et al., 2015; Viveros et al., 2005; Moreira et al., 2008).

The overall aim of this thesis was to find whether there is an analgesic oxycodone concentration and to determine more precise, predictive factors to estimate the oxycodone doses needed for satisfactory post-operative analgesia. More specifically, the aim was to clarify the roles of important clinical variables, *OPRM1* rs1799971 and *FAAH* polymorphisms on analgesic post-operative dose and plasma concentration of oxycodone.

2. REVIEW OF LITERATURE

2.1 Pain

According to the International Association for the Study of Pain, pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP Taxonomy 2018). Pain is not only a vital protective signal to prevent tissue damage but a stimulus to teach the avoidance of harmful behaviour. Pain can be categorized in many ways; based on duration, pain can be defined as acute (less than 6 weeks), subacute (6 weeks to 3 months) or chronic (more than 3 months). The type of pain can be categorized as nociceptive, neuropathic or inflammatory. Several other classification methods exist and even more ways are used to describe the sensation pain causes. Nevertheless, pain is always a subjective experience and for instance, individuals report highly variable pain scores even when the nociceptive stimulus has been standardized (Coghill et al., 2003). The sensation caused by a nociceptive stimulus is a complex phenomenon, which is affected by many biological and psychological factors.

2.1.1 From nociception to pain

The human body can detect many somatosensory stimuli such as touch, vibration, temperature, proprioception and nociception. Nociception is the peripheral nervous system's response to certain harmful or potentially harmful stimuli, which is usually experienced as pain. The pain experience generally starts with the activation of a peripheral nerve ending called the nociceptor. Nociceptors can be activated by multiple factors such as temperature, pressure and chemical agents. A stimulus strong enough in intensity causes the cation channels on a nociceptor to open, and the cell membrane depolarizes. If a threshold potential is achieved, an action potential is created, and the signal starts to move towards the CNS. Most often neurons conducting nociceptive stimuli are either Aδ or C-fibres. Aδ fibres are small, myelinated, rapidly conducting neurons and their activation quickly causes the sensation of sharp, easily localized pain. C-fibres are small, unmyelinated neurons with much broader sensory areas than the Aδ fibres. C-fibre activation leads to the slower sensation of dull, burning pain (Basbaum et al. 2009).

The cell bodies of the nociceptive neurons (or primary afferents neurons of the pain pathway) are located in the dorsal root ganglia. From there they project to the dorsal horn of the spinal cord where the primary afferent neurons synapse with the secondary afferent neurons of the pain pathway. The nociceptive impulse ascends through the spinothalamic and spinoreticular tracts to the thalamus. From the thalamus, tertiary

afferent neurons convey the stimulus forward to higher centres of the CNS such as anterior cingulate cortex, somatosensory cortex and prefrontal cortex (Steeds, 2009) (Figure 1).

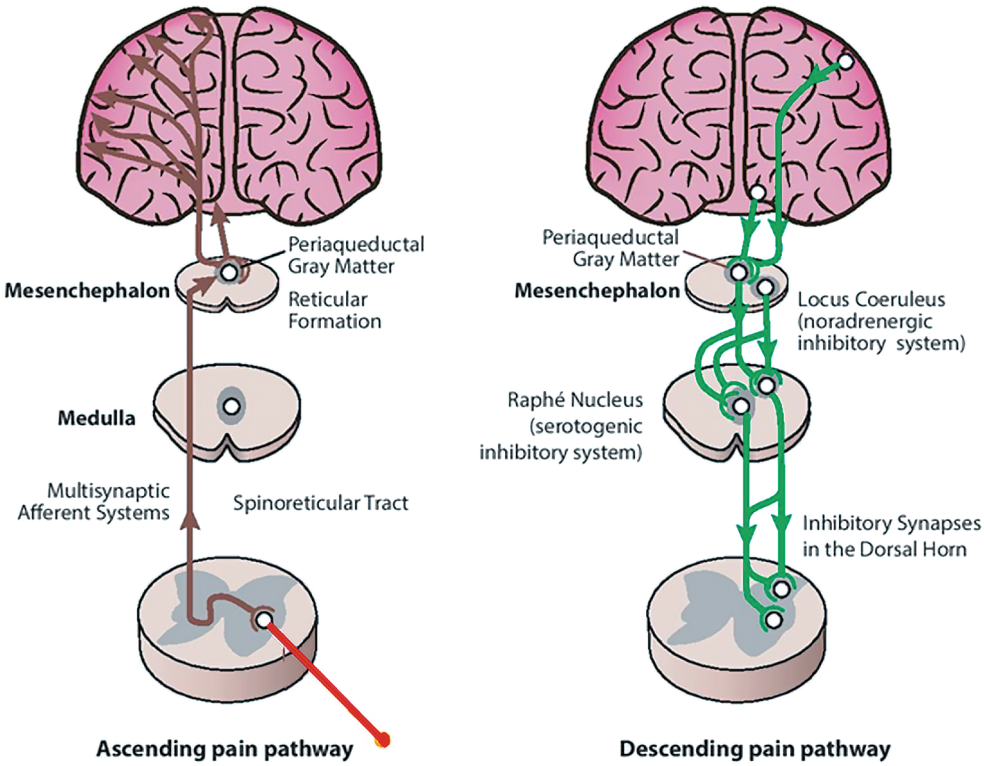

Figure 1. Ascending spinoreticular tract and descending pain pathways. Afferent pain pathway ascends towards the thalamus and cortex. Primary afferent neuron marked in red. Secondary and tertiary afferents marked in brown. Descending pain pathway is activated by the pain sensation. Neurons in descending pain pathway synapse with the ascending pain pathway on many levels modifying the pain experience.

The pain signal is highly modulated before the actual pain sensation occurs. Pain modulation is conveyed back to the dorsal horn of the spinal cord via descending pain pathways, which can be either inhibitory or excitatory. When an individual experiences pain, periaqueductal grey (PAG) starts to receive input from the higher brain areas. This activates PAG that in turn, further activates the cells located on the rostroventromedial medulla (RVM). Different parts of RVM are activated in descending pain facilitation and inhibition. Studies have recognized both OFF-cells that activate the descending inhibitory pain pathway and ON-cells, which activate the facilitatory pathways. The presence of both cell types provides a cellular explanation for the pain modulation by higher cortical areas. Opioids inhibit ON-cells and excite OFF-cells (Ossipov et al., 2014). The pain signal can be inhibited in the nuclei of RVM, but the descending pain

pathway also projects downwards through the nucleus raphe magnus and finally to the dorsal horn of the spinal cord. Neurons in the inhibitory descending pain pathway release serotonin, norepinephrine and other neurotransmitters that inhibit the activation of the cells in the ascending pain pathway (Figure 1).

Acute pain is an important protective signal that prevents the individual from harming their body in a possibly lethal way. When a painful sensation continues for an extended time-period, it loses its protective nature and becomes a burden for the individual. Pain is usually defined as chronic if it has lasted for more than three months or longer than the expected recovery from a tissue injury would normally take. In chronic pain, the pain the function of the pain sensing system is altered. The pathology behind a chronic pain condition can be either in the peripheral nerves, CNS or even in multiple locations at the same time. In peripheral nerve damage, the injured nerve can be sensitized to many normally non-painful stimuli. In the CNS, chronic pain can be a sum of many changes. Chronic pain patients usually express higher sensitivity to pain. Recently, increased interest has focused on the function of the descending pain system in chronic pain patients. MRI and fMRI imaging has shown anatomical and functional differences, such as abnormal white matter connectivity and regional grey matter atrophy, between chronic pain patients and healthy controls (Geha et al., 2008; Baliki et al., 2008).

2.1.2 Measuring pain

Pain is always an individual experience composed of sensory-discriminative, affective, cognitive and behavioural components. Nonetheless, in order to treat and study pain, several methods for pain assessment have been developed. Many of these tools are relatively simple and focus on the intensity/unpleasantness of pain. One such a tool is the Visual Analog Scale (VAS) that comprises a 100 mm line segment. With VAS the patient is asked to indicate his/her pain intensity on the line, 0 mm indicating no pain and 100 mm indicating the worst pain possible. Additionally, VAS can also have graphic designs to help patients better interpret the line like colours, numbers, faces indicating different pain intensities and a rising shape. VAS is widely used because of the simplicity and convenience of the test. Moreover, the test is reliable – although it is not suitable for cognitively impaired people (Hawker et al., 2011) – and it has been validated using fMRI imaging (Coghill et al., 2003).

Another broadly used pain-measuring tool is the Numerical Rating Scale (NRS). This test does not have a visual aspect like VAS, but the patient is rather asked to evaluate the pain intensity with a number. Generally, the range from zero to 10 is used with zero indicating no pain and 10 the worst pain imaginable. NRS scores of 1-3 are considered mild, 4-7 moderate and 8-10 severe pain. Overall, the reliability of the test is similar to that of VAS (Hawker et al., 2011).

There is a multitude of questionnaires for assessing chronic pain. The most common ones include the McGill Pain Score - that focuses on intensity, affective, sensory and evaluative aspects of pain and the Brief Pain Inventory - which puts emphases on the location of pain and how it interferes with various aspects of life (Cleeland et al. 1991; Melzack, 1975; Daut, 1983). While the completion of VAS and NRS only takes seconds, these questionnaires require more time, but in return provide a much broader assessment of pain type (Hawker et al., 2011).

2.1.3 Thermal pain sensitivity

Cold sensation is mainly mediated by the TRPM8 belonging to the Transient Receptor Potential family, also known as cold- and menthol-sensitive receptor 1 (Bautista et al., 2007). This receptor is activated at skin temperatures below 26 °C, whereas below 10 °C it becomes inactive as the skin temperature falls to the point where cold starts to act as a numbing agent (Peier et al., 2002). In addition to temperatures below 26 °C, a number of chemical agents including menthol can activate this receptor. TRPM8 receptors are Ca^{2+} permeable, non-selective cations channels expressed on the surface of C-fibres. There is also evidence that cold sensation can occur through other mechanisms besides the activation of TRPM8. For example, *TRPM8 -/-* mice detected cold stimuli but at lower temperatures than wild-type mice. Moreover, *TRPM8 -/-* mice also tolerated noxious cold stimuli for a longer time than their wild-type counterparts (Bautista et al., 2007). Additionally, TRPA1 receptor can be a receptor for noxious cold stimuli, but so far, studies have been inconclusive of its role (Kim et al., 2006; Knowlton et al., 2010; Schutz et al., 2014).

Heat pain perception is also caused by the activation of a multitude of receptors belonging to the Transient Receptor Potential family. The most studied of these is the TRPV1 receptor, which senses capsaicin induced heat sensation. Other known heat pain receptors include TRPV2, TRPV3 and TRPV4 (Venkatachalam et al, 2007) which all activate at different temperatures to provide an individual with a sense of heat intensity. TRPV1 is known to activate at a temperature of 43 °C while, for example, TRPV2 is only activated at temperatures above 52 °C (Caterina et al., 1999) (Table 1).

Table 1. Receptors involved in thermosensation.

TRPV1	uncomfortably warm	>43 °C	Caterina et al., 1999
TRPV2	noxious heat	>52 °C	Caterina et al., 1999
TRPV3	innocuous heat	30-39 °C	Peier et al. 2002
TRPV4	innocuous heat	25-34 °C	Watanabe et al., 2002
TRPM8	innocuous cold	<26 °C	Peier et al. 2002
TRPA1	noxious cold (not verified)		

The cell bodies of these thermosensory cells locate in the trigeminal ganglia and dorsal root ganglia. Centrally they project to the lamina I and outer layer of lamina II of the dorsal horn of the spinal cord. Temperature signals reach the brain via the lateral spinothalamic tract, while noxious signals are additionally carried via the spinoreticular tract (Almeida et al., 2004; Willis et al., 1997; Giordano 2005). In healthy humans, stimulation of the forearm skin with innocuous cold leads to activation of the contra- and ipsilateral-posterior insular cortices and the primary somatosensory area. In contrast, noxious cold stimuli activate the contra- and ipsilateral-insular cortices, secondary somatosensory cortices and the cingulate cortex (Maihöfner et al., 2002).

Cold pain thresholds vary much more between individuals than hot pain thresholds. Furthermore, patients also describe the cold pain sensation with a wide range of qualitative words, whereas heat pain is typically defined as burning (Morin et al., 1998). Interestingly, the cold pain threshold has also been shown to remain relatively steady when the same individual is retested (Moss et al., 2016).

Experimental pain sensitivity has been hypothesised to predict the postoperative pain outcomes and act as a tool for clinicians to predict which patients require more analgesics after surgery. So far, studies have been inconclusive on whether experimental pain measurements have any clinical significance. A recent review showed that in spite of extensive research, no consistent association between experimental pain variables and pain after surgery exists (Sangesland et al., 2017).

Although other nociceptive stimuli - like chemical and mechanic - are known to exist in addition to thermal stimuli, these are not addressed within this thesis.

2.1.4 Postoperative pain

The intensity of postoperative pain depends on many aspects including patient and surgery-dependent factors. The characteristics of the operation predispose patients to different types and intensities of pain depending on the invasiveness of the surgery, the tissue being operated on and the site of the operation. Post-operative pain can comprise of only one type of pain, but it can also consist of a mixture of nociceptive, neuropathic and visceral pain.

Traditionally, greater pain intensities are expected after surgeries that are highly invasive, especially in areas highly innervated with sensory nerve fibres. However, a study by Gerbershagen et al. showed that many minor, everyday procedures resulted in surprisingly high postoperative pain intensities. Such types of surgeries included appendectomy, haemorrhoidectomy and tonsillectomy, which ranked among the 25 operations with the highest post-operative pain intensities. Of the 25 operations with highest postoperative pain intensities, 17 were trauma/orthopaedic and five abdominal surgery procedures. With regard to minor procedures, a key factor for the surprisingly high pain intensities may have been insufficient administration of analgesics. The post-

operative morphine equivalents administered after these operations were much lower than for the more extensive surgeries, even though the patients reported high post-operative pain intensities (Gerbershagen et al., 2013).

With the exception of the type of surgery, not many surgery-related related predictive factors of post-operative pain intensities have been identified. Intraoperative opioid use or preoperative patient education about surgery showed no association to post-operative pain intensity. Additionally, results on the correlation between incision size, duration of the surgery, number of previous surgeries and post-operative pain have been inconclusive (Ip et al., 2009).

Many patient-related factors affecting postoperative pain intensities are known. In our cohort factors associated with postoperative pain intensities in prior studies include type of surgery (breast conserving/mastectomy with sentinel node biopsy/ axillary clearance), age and experimental heat pain intensity (Kaunisto et al., 2013).

One of the most widely studied factors affecting postoperative pain is anxiety, which has been shown to increase perceived pain intensities and post-operative analgesic requirements (Hui et al., 2009). Interestingly, preoperative diazepam has been found not to affect post-operative pain outcomes or analgesic requirements (Caumo et al., 2002). Other psychological factors shown to affect postoperative pain intensities are the expectance of pain, depressed mood/negative affect, pain self-distraction and catastrophizing as a coping mechanism and personality traits (for example neuroticism, hostility) (Sipilä et al., 2017; Ip et al., 2009).

Age has also been shown to decrease both post-operative pain intensities and analgesic consumption. With advancing age, the nervous system is exposed to many degenerative processes such as atrophy in the cortex, changes in neurotransmitter levels and the density of peripheral nerve fibres (Fjell et al., 2009; Ko et al., 1997. Verdú et al., 2000). All these processes change how pain is perceived. The changes in analgesic requirement have been hypothesised to be due to a lower percentage of body water as well as decreased liver and kidney function, which alters both the metabolism and excretion of analgesics (Liukas et al., 2011; Saari et al., 2012).

The effect of gender on postoperative pain is still under dispute. Some studies have found an association between the female sex and higher pain intensities after surgery (Zheng et al., 2017) whilst others have failed to find any association, which might be due to the relatively small effect of gender on post-operative pain (Ip et al., 2009). Possible reasons for this difference have been hypothesised to be lower endogenous pain inhibition in women as well as differences in central sensitization, hormonal differences and social aspects toward pain behaviour (Pereira et al., 2015).

The effect of BMI on post-operative pain intensity is also unclear. Only a few studies have addressed the effect of BMI to post-operative pain. One study found association with increased postoperative pain intensities and high BMI, whilst

another determined no association (Ip et al., 2009). In our cohort, BMI appeared not to affect post-operative pain intensity (Kaunisto et al., 2013).

Tobacco smoking has been shown to associate with higher postoperative pain intensities and an increase in opioid consumption (Bortoluzzi et al., 2012; Chiang et al., 2016; Yang et al., 2012). Although the mechanism that results in these pain intensity differences remains unknown, it could be partially due to chronic inflammation processes, deficient wound healing and a higher occurrence of complications (Myles et al., 2002; Sorensen et al., 2005; Lewin et al., 2014). The reason for the association between tobacco smoking and opioid requirements is unclear, but it is thought to be due to pharmacokinetic or demographic factors (Shi et al., 2010). In our study sample, previous smoking was associated with decreased experimental heat pain sensitivity but otherwise smoking did not affect pain intensities or oxycodone consumption (Kaunisto et al., 2013).

2.1.5 Management of post-operative pain

Modern day surgery requires efficient analgesia and good pain management helps the body to cope with the stress caused by surgery and enhances recovery. Opioids are the gold standard analgesic for moderate to severe acute postoperative pain. Often the pain management protocol starts before anaesthesia with premedication (usually NSAID or paracetamol). Induction of anaesthesia begins with an intravenous (i.v.) dose of a rapidly acting opioid such as remifentanil or fentanyl. During surgery, more opioid is administered if the patient shows signs of pain, such as an increased heart rate. Before reversing anaesthesia, the wound itself is often anaesthetised with a local anaesthetic to reduce nociceptive pain.

In the post-operative care unit (PACU) patients are usually given i.v. opioids with dosing adjusted in relation to the patient's age, weight and pain intensity. The goal is that the pain intensity is no more than mild (NRS 0-3). In the PACU, patients are monitored intensively and once they have recovered from the anaesthesia and symptoms (e.g. pain and nausea) are under control they can be moved to the ward or in case of minor surgeries, discharged. In the ward, patients receive analgesics either with a PCA or by oral administration. Usually NSAID or paracetamol is used as a base analgesic and opioid is only administered if needed. Patients must still be monitored and pain intensity evaluated regularly to ensure adequate pain relief.

2.2 Opioids

Opioids are a group of drugs that interact with G-protein coupled opioid receptors (mu, kappa, and delta). Endogenous opioid system is a vital part of the central and peripheral neurotransmitter networks. Endogenous opioids include i.e. endorphins, dynorphins, enkephalins and endomorphins. Exogenous opioids, like morphine,

oxycodone and fentanyl, find use mainly as analgesics for moderate and severe acute pain e.g. after surgery or trauma, and cancer pain. Currently the opinion is opioids produce analgesia mainly by activating the mu-opioid receptors in the CNS.

Opioid receptors are highly expressed in subcortical regions of the CNS such as brainstem, midbrain periaqueductal grey, ventral tegmental area, nucleus raphe magnus, the rostral ventral medulla, hypothalamus, amygdala, thalamus and cortex, from which descending pain pathways originate. Opioids are thought to activate the descending pain pathways through the disinhibition of GABAergic interneurons, which leads to activation of descending neurons that inhibit signal transmission in the spinal cord.

In the spinal cord opioid receptors can be found on the surface of pre- and postsynaptic cell membranes as well as interneurons. Opioids produce analgesia by inhibiting spinal nociceptive transmission. The activation of opioid receptors on the presynaptic neuron reduces cAMP signalling and decreases the activity of voltage-gated calcium channels. This inhibits the release of neurotransmitters to the synaptic cleft thus preventing the pain signal transmission. The activation of opioid receptors located on the postsynaptic cell membrane causes hyperpolarization by activating the potassium influx channels (Trang et al., 2015).

2.2.1 Oxycodone

Synthesized in 1917 in Germany, this semi-synthetic opioid has replaced morphine as an analgesic due to higher oral bioavailability, more rapid penetration through the blood-brain-barrier, longer duration of action and less side effects (Olkkola el al., 2013). Oxycodone is derived from codeine and the chemical structures of oxycodone and codeine are quite similar with exception that oxycodone has one extra carbonyl and hydroxyl groups and lacks a double bond (Figure 2). Oxycodone is used increasingly worldwide for moderate and severe acute pain as well as chronic pain conditions. In Finland, oxycodone has been the most used post-operative opioid analgesic since the 1970s.

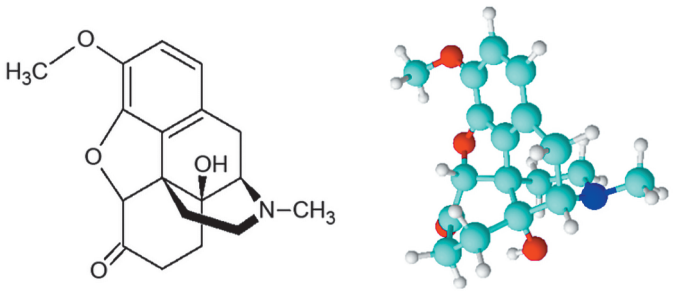

Figure 2. The chemical structure of oxycodone in 2D and 3D

2.2.1.1 Pharmacokinetics

When administered intravenously, the analgesic effect of oxycodone commences after 5-8 minutes (Leow et al., 1995), whereas after oral administration, the analgesic effects are noticed after 10-30min. Oxycodone has a relatively high oral bioavailability of 60-80%, which greatly exceeds that of morphine (15-40%) and additionally the interindividual variation in bioavailability is smaller with oxycodone than morphine, making it easier to administer. The peak plasma concentration of oxycodone is reached after 1.5-2 hours when administered as oral solution. Oxycodone is more hydrophopic than morphine. About 40% of oxycodone is bound to plasma proteins when studied *in vitro* and the elimination half-life for oxycodone is 3-5h (Olkkola et al., 2013).

Oxycodone is metabolized primarily by CYP3A4/5 and CYP2D6 enzymes located in enterocytes of the small intestine and hepatocytes with the main metabolic pathway involving N-demethylation to noroxycodone via CYP3A4. Noroxycodone has a weak opioid potency and is thought have no major role in oxycodone-induced analgesia. However, O-demethylation by CYP2D6 yields oxymorphone - a strong mu-opioid receptor agonist - that has been suggested to play a role in oxycodone analgesia. The affinity of oxymorphone to the mu-opioid receptor could be up to 40-times higher than that of oxycodone (Lalovic et al., 2006). So far, the data regarding the clinical significance of oxymorphone has been inconclusive since the concentration of the metabolite remains low in plasma and possibly in the CNS as well (Heiskanen et al. 1998). Both noroxycodone and oxymorphone are further metabolised to noroxymorphone by CYP3A4/5 and CYP2D6, respectively. Noroxymorphone has about two to three times higher affinity to the mu-opioid receptor than oxycodone, nonetheless, it penetrates the BBB poorly and therefore has mainly peripheral effects (Lalovic et al., 2006). A minor metabolic pathway of ketone reduction yields two stereoisomers of oxycodol from oxycodone, noroxycodol from noroxycodone, and oxymorphol from oxymorphone (Figure 3.).

There is evidence that oxycodone accumulates in the CNS. Though no human data exist, studies in rats has shown the brain-plasma ratio to be 2:1 (Lalovic et al., 2006). This was hypothesized to be due to active transport through the BBB (Bostrom et al., 2008), as oxycodone has been shown to cross the BBB up to 7 times faster than morphine (Villesen et al., 2006). So far, the most promising candidate for this protein has been the pyrilamine transporter that has been shown to partly mediate transportation of oxycodone to the CNS in rats. No human data for this or any other transporter is available. The effect of P-glycoprotein on the CSF concentrations of oxycodone has been debated. Initially, P-glycoprotein was thought to have no role in the pharmacokinetics of oxycodone, though later it was demonstrated that oxycodone, like morphine, acts as a substrate for the P-glycoprotein and therefore is possibly subjected to active efflux transportation (Hassan et al. 2007).

Oxycodone and its metabolites are all excreted through the kidneys either in a conjugated or unconjugated form. Both renal and hepatic impairment influence the maximum plasma concentrations and the effect time of oxycodone. In a study published in 2015, severe renal impairment almost doubled the maximum plasma concentration after a 10mg oral dose (Malhotra et al., 2015). The AUC from detection to last detectable concentration rose 1.5 times with moderate renal function and more than doubled with severely impaired kidney function when compared to normal kidney function. Similarly, moderate hepatic impairment doubled the maximum plasma concentration and the AUC after a single dose of 10mg oxycodone when compared to normal hepatic function (Malhotra et al., 2015).

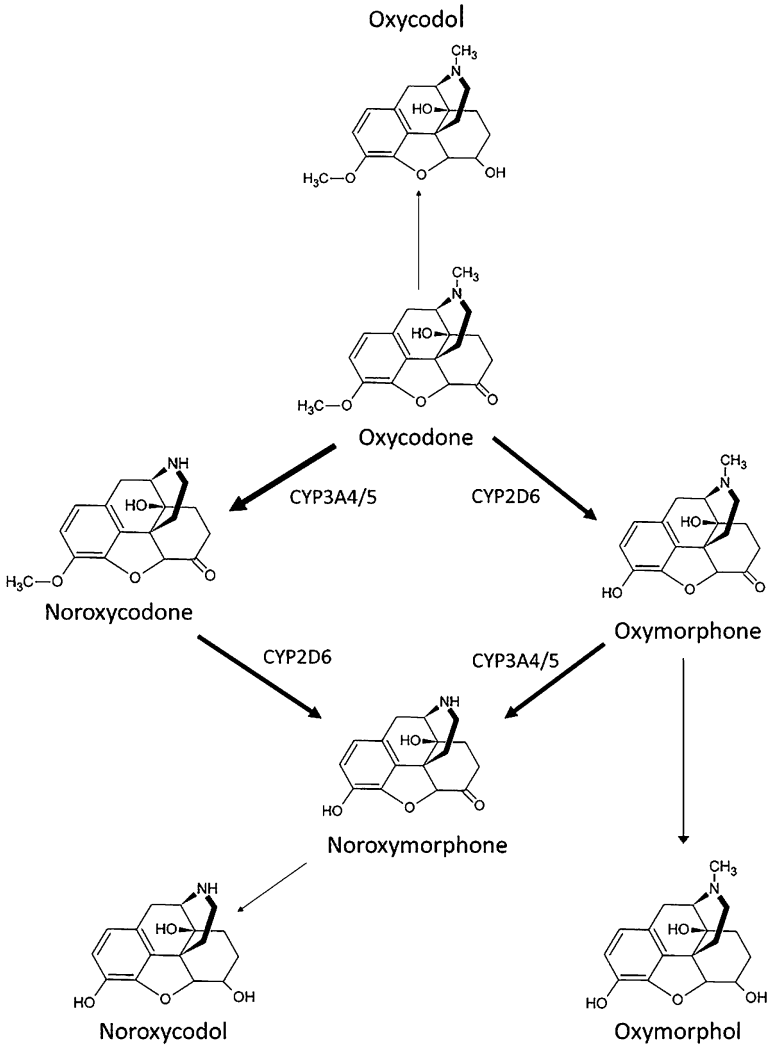

Figure 3. The metabolic pathways of oxycodone

2.2.1.2 Pharmacodynamics

Oxycodone is a relatively selective mu-opioid agonist, but it does bind to kappa- and delta-receptors to some extent. The binding affinity of oxycodone to mu-opioid receptor is 5-40 times lower when compared with morphine (Lalovic et al., 2006; Lemberg et al., 2006). In addition, oxycodone also activates 4-8 times less intracellular G proteins than morphine (Lalovic et al., 2006). There is evidence that oxycodone G protein activating potency varies in different parts of the CNS and that this potency can even be altered by different disease states such as bone cancer (Nakamura et al., 2013).

Besides analgesia, oxycodone also causes decreased alertness, nausea, changes in mood, pruritus and pupil constriction (Lalovic et al., 2006). Oxycodone, like other opioids, also has antitussive effects and can cause respiratory depression (Chang et al., 2010). Furthermore, other known side effects include constipation, urinary retention and sweating (Kalso et al., 1990).

2.2.2 Studying the analgesic effect of opioids: dose vs. plasma concentration

When studying the analgesic effects of opioids in humans, the most utilized measure is the dose: Either the analgesic effect of a fixed dose is determined or the dose required to achieve a pre-set analgesia status is measured. Opioid dose can be monitored easily, which makes it a tempting variable for research. Nevertheless, as the final effect of opioids is conveyed by the mu-opioid receptors in the CNS, many pharmacokinetic events can alter the amount of opioid reaching the mu-opioid receptor before any analgesia can occur. Therefore, there is always a degree of inaccuracy when determining analgesia from the dose required.

In order to achieve the best accuracy to estimate how much opioid is needed to activate the opioid receptors, measurement of CSF concentrations would be the favoured clinical method. Two studies have addressed the difference in analgesic doses between epidurally and intravenously administered oxycodone (Backlund et al., 1997; Kokki et al., 2014). In the study by Kokki et al. the amount of oxycodone administered was standardized and no analgesic CSF concentration could be determined, whereas in the research of Bäcklund et al. oxycodone concentrations were only measured from the plasma. Measurement of the CSF concentration of any opioid requires insertion of a spinal catheter and frequent sample collection must be performed. Furthermore, the procedure is invasive and includes a risk for headache and a minor risk for infection.

2.3 Genetics

Genetics is the study of the science of heredity, dealing with resemblances and differences of related organisms resulting from the interaction of their genes and the environment.

The human genome consists of double-stranded deoxyribonucleic acid (DNA) which forms the genetic code of an individual. The human genome is estimated to have around 20,000-25,000 genes (International Human Sequencing Consortium, 2004). These comprise only a fraction of the complete human genome even when all the areas regulatory regions are included. In addition, the genome contains many areas that code for non-protein molecules like micro-RNAs. Most of the genome consist of DNA-areas with unknown function (The ENCODE Project Consortium, 2012).

Each gene has regulatory areas normally located upstream from the coding region and these usually contain a number of enhancer/silencer sequences and promoter sequences. The enhancer/silencer sequences can alter the transcription activity of the gene. The gene itself consist of exon and intron sequences: Intron sequences are transcribed to the premature messenger RNA (mRNA) but are then removed by splicing before translation. In contrast, the exons are first transcribed to mRNA, before later being translated to proteins.

The individuality of each person's genetic makeup comes from DNA polymorphism, which is defined as the occurrence of two or more alleles at one locus in the same population, each with appreciable frequency (Cavalli-Sforza et al., 1971). Some common types of genetic polymorphism are listed in Table 2.

Table 2. Common types of genetic polymorphisms. Bp = base pair, kb = kilo base pair (1,000 base pairs), Mb = mega base pair (1,000,000 base pairs). Modified from Genomic and personalized medicine, Chapter 1 (Willard 2013)

Type of polymorphism	Change	Possible effect
Single nucleotide polymorphism	Alters one nucleotide	·Transcription +/- ·Protein configuration alteration ·Often no effect
Insertion / deletion	Inserts or deletes a sequence of DNA sized 1 bp - 10 kb	·Frameshift mutation ·Protein configuration alteration ·Often no effect
Copy number variants	Repeats a sequence sized 1 kb - 1Mb in the DNA strand	·Transcription +/-
Inversion	A part of DNA breaks loose, rotates 180° and attaches back in the original place	·Often no effect ·Transcription +/- ·Protein configuration alteration

In genetic studies, the individual's genetic code or genotype of certain polymorphic loci must be determined. This can be done in various extents. When a specific candidate polymorphism or gene is hypothesized to have an association with the studied phenotype, only this part of genome needs to be analysed by sequencing or genotyping methods. If the genome phenotype interaction needs to be examined on a larger scale, genome-wide SNP genotyping, exome or genome sequencing can be used. In the case of genome sequencing, the nucleotide order of the whole genome is determined, while in exome sequencing only the protein coding parts of the DNA are sequenced. Exons only comprise about 1% of the genome so exome sequencing produces significantly less data than genome sequencing. When looking at polymorphism associations with a studied phenotype on a genome wide scale, the alleles of hundreds of thousands to millions of previously known polymorphisms are determined from the study population, after which a genome wide association study (GWAS) is performed. GWAS has made it possible to explore genomic-phenotype associations on a large scale. The method is best used for diseases with multiple effecting genes, since GWAS has poor recognition of rare variants or polymorphisms with low allele frequencies. GWAS requires a large sample size since the effect of individual genetic variants on the studied phenotype is usually very small. In addition, any associations found must also be replicated in another cohort and preferably studied with targeted gene sequencing before clinical significance can be determined (Bush et al., 2012).

In practise, associations between a genotype and certain phenotype are determined by comparing allele or genotype frequencies between cases of interest and appropriate controls. If the allelic frequency is much higher in case-group, there is a positive association to the studied phenotype. For continuous phenotypes (e.g. height), the mean values of each genotype are compared and if the means of the studied phenotype vary significantly between the different genotypes, an association is present. Nevertheless, the final interpretation of the data can be difficult, especially as the case and control populations should be matched for ancestry. Furthermore, an association does not equal causation as an association between a polymorphism and phenotype is only a starting point when studying the effect of genetics on a clinical phenotype.

When a rare, high-impact variant is expected to cause the studied phenotype, sequencing methods are used. Previously, targeted Sanger sequencing of a specific gene was frequently used due to high-cost of sequencing and such targeted sequencing can be quite effective in recognising rare variants as it allows a high depth of coverage. Nonetheless, in order to utilize this method, a candidate mutation or gene first must be assumed to exist. Moreover, targeted sequencing also only produces data from the chosen area so many important variations can be missed. As the cost of sequencing has significantly decreased in recent years, it is now possible to use exome and genome sequencing. Exome and genome sequencing look at the genetic code on a wider scale making it possible to observe more gene-phenotype associations. The drawback to genome sequencing however - and to some extend

with exome sequencing - is that these methods produce a significant amount of data, which is often quite hard to interpret since every individual's genome contains tens of thousands of rare variations with unknown significance (Tennesen et al., 2012; Nelson et al., 2012). Consequently, the analysis of the sequenced data generally targets a few specific areas of interest.

2.3.1 Genetics of pain and analgesia

2.3.1.1 Genetics of pain

The individuality of pain sensitivity and analgesic requirements have raised the question as to the role of genetics in pain susceptibility and effectiveness of analgesics. Multiple studies have examined potential gene polymorphism in order to identify the aetiology behind this interindividual variability. Several genetic variants have been shown to be associated with pain intensity, threshold and opioid requirements, but so far, their effect has been small when compared with known phenotype-phenotype interactions like pain intensity and anxiety.

Heritability of pain varies greatly and there are a number of rare pain syndromes that are caused by mutation in a single gene. Usually these single-gene disorders result in dramatic phenotypes, like in the case of HSAN2 syndrome caused by missense mutations in the protein kinase PRKWNK1 that results in an individual's total loss of all sensation. In addition, mutations in the sodium channel NaV1.7 coding *SCN9A* have also been determined to cause multiple genetic pain disorders (Dreth et al., 2007). In addition to rare pain syndromes, a common genetic background has been shown to affect pain sensitivity with most experimental pain types. Studies conducted with monozygotic and dizygotic twins have shown the genetic component of different experimental pain intensities and thresholds can explain 22% to 60% of the variance observed (Norbury et al., 2007; Nielsen et al., 2008).

Genes can potentially affect pain intensity at any point of the pain pathway. An example for peripheral effect is the *TRPA1* gene coding for TRPA1 receptor. *TRPA1* rs1198795 has been shown to alter reported cold pain intensity significantly (Kim 2006). Pain processing occurs also higher up in the pain pathway with examples of genes expressed in the CNS that are hypothesized to affect pain intensities are *OPRM1, FAAH* and *COMT*.

Genetics of different pain phenotypes have been extensively studied, but thus far only one small-scale exome sequencing study focusing on experimental heat pain has been performed, which resulted in no clear findings. The most significant associated gene was *GZMM* which codes for serine protease protein expressed in natural killer cells and lymphocytes. A later network analysis also showed an association between angiotensin II network and heat pain sensitivity (Williams et al.,

2012). There is previous evidence that angiotensin II can affect pain, but currently the results in humans have been conflicting (Kalra et al., 2008; Guasti et al., 2002). Other studies, which take the candidate gene approach, have found association between experimental pain and *COMT*, *OPRM1*, *FAAH*, *GCH1*, *MC1R* and *TRPA1* variants (Kim et al., 2009a).

Acute post-operative pain has been addressed in one GWAS. In this study, the main studied phenotype was analgesia onset after NSAID administration and results showed that there were no significant associations to post-operative pain intensity. (Kim et al., 2009b). In addition to the GWAS, there have also been studies that investigated potential genes associated with post-operative pain (Kim et al., 2009a).

2.3.1.2 Pharmacogenetics of analgetics

Pharmacogenetics is the scientific speciality studying the drug response variability due to genetic factors. Genetic polymorphism can affect the drug response on many levels, for example, genetic variants can affect how the drug is absorbed, metabolized, bound to proteins and secreted from the body or change the pharmacodynamics of the drug. Significant drug-genotype interactions have already been revealed, such as the effect of *CYP2D6* polymorphisms on codeine metabolism to morphine (Dayer et al., 1988). This has been demonstrated by a reported incident where paediatric patient experienced morphine overdose after codeine administration due to a version of the CYP2D6 enzyme converting codeine to morphine much more rapidly than expected (Orliaguet, 2015). Currently a gene test to determine CYP2D6 genotype is used clinically to evaluate the dose and duration of action of a multitude of drugs.

Post-operative opioid requirement varies significantly between individuals. Many potential genetic contributors to this variance have been studied, but to date the results have been either conflicting or the associations weak. In a recent GWAS Cook-Sather et al. found multiple nominally significant associations between SNPs in the *TAOK3*, *TEC* and *MFI2* gene regions and post-operative morphine requirements in a paediatric surgery population (Cook-Sather et al., 2014).

2.3.2 Genes possibly affecting postoperative pain and analgesia

2.3.2.1 OPRM1

OPRM1, located on the long arm of chromosome 6, is the gene coding for the mu-opioid receptor. The gene spans around 230,000 base pairs and contains 17 exons. OPRM1 is undergoes extensive alternative splicing and several of alternatively spliced variants have been identified. *OPRM1* is primarily expressed in the CNS, but also in the adrenal gland, thyroid gland and testis (NCBI Gene Database: *OPRM1*).

Of the more than three thousand known polymorphisms in the *OPRM1,* rs1799971, (g.[118A>G]) is of special interest. This polymorphism changes aspargine 40 to aspartate 40 (p.[Asn40Asp]), therefore altering the configuration of the final receptor protein. This change in the amino acid sequence of the extracellular N-terminal end leads to a loss of a glycosylation site, which has been determined to destabilize the receptor. The half-life of the Asp^{40} (mutated) receptor was only 11.6 h compared to 27.7 h for Asn^{40} (wild-type) receptor (Huang et al., 2012). Rs1799971 polymorphism has also been shown to alter the receptor's binding potential to β-endorphin and possibly to other opioids, as well as mRNA expression (Bond et al., 1998; Zhang et al., 2005), which could change the downstream signalling caused by opioids binding to the receptor.

OPRM1 rs1799971 has been extensively associated with many phenotypes (Table 4). The effects of rs1799971 on opioid requirements and pain intensity have been researched extensively, particularly with morphine or fentanyl. Prior to this study, only two studies have looked at the association between oxycodone requirement and rs1799971. (Bruehl et al., 2006; Zwisler et al., 2012) (Table 3.).

Many of the studies that examine *OPRM1* rs1799971 polymorphism's effect on post-operative pain and opioid requirements have been performed with relatively small patient groups (50-200 patients) and showed variable results. The three largest studies conducted with more than 500 patients showed that G-allele carriers required more opioids post-operatively and also reported higher post-operative pain scores (Tan et al., 2009; Sia et al.,2013; Sia et al., 2008) (Table 3.). A recent review article and meta-analysis concluded that rs1799971 does affect the post-operative pain intensities and opioid requirements during the first 24h but that this effect wears off before 48h has passed (Ren et al., 2015). The analysis was performed by comparing major allele homozygote (AA) individuals with G-allele carriers, so the possible differences between heterozygous and minor allele homozygous (GG) individuals could not be estimated. There is evidence that GG individuals would require more opioids when compared to patients who were heterozygous for the polymorphism (Zhang et al., 2010; Zhang et al., 2011; Fukuda et al., 2010).

The allele frequency of the *OPRM1* polymorphism varies greatly in different populations. G-allele frequency is highest among Japanese (48%) and most rare among African populations from Nigeria, Gambia and Sierra Leone (0%). In Finland, the frequency of G-allele is about 20%, which is similar to other Northern European populations (NCBI Variation Viewer, Sequencing Initiative Suomi Project (SISu)). When the gene's effect in different ethnic groups was studied, it emerged that the gene may alter experimental pain intensity differently in people with different ancestry (Hastie et al., 2012). Nevertheless, there are currently no studies that investigate the combined effect of ethnicity and *OPRM1* rs1799971 on postoperative pain intensity or opioid requirement. However, in many studies both ethnicity and rs1799971 polymorphism have been shown to be significant independent factors (Tan et al., 2009; Somogyi et al., 2016)

Table 3. Studies assessing OPRM1 rs1799971 and postoperative pain intensity and opioid consumption. A = Asian, I = Indian, C = Caucasian, M = multiple.

						OPRM1 rs1799971 Genotype			Effect of OPRM1 rs 1799971	
Article	**Number of patients**	**Population**	**Operation**	**Opioid**	**MAF**	**AA**	**AG**	**GG**	**Postoperative Pain Intensity**	**Postoperative Oxycodone Consumption**
Tan et al 2009	994	A, I	Caesarean section	morphine	0.39	389	435	170	increase	increase
Sia et al 2013	973	A, I	Hysterectomy	morphine	0.39	354	474	145	increase	increase
Sia et al 2008	585	A	Caesarean section	morphine	0.34	271	243	80	increase	increase
Fukuda et al 2009	280	A	Orofacial surgery	fentanyl	0.44	86	143	51	no effect	no effect
Zwisler et al 2012	266	C	Thyroidectomy and others	oxycodone	0.10	219	43	4	no effect	no effect
De Gregori et al 2016	201	C	Major abdominal surgery	morphine	0.18	136	56	9	no effect	increase
Kim et al 2013	196	A	Hysterectomy	fentanyl	0.39	72	96	28	n/a	no effect
Zhang et al 2010	174	A	Hysterectomy or myomectomy	fentanyl	0.31	86	67	21	no effect	increase
Zhang et al 2011	165	A	Hysterectomy or myomectomy	fentanyl	0.32	80	63	22	no effect	increase
Hayashida et al 2008	138	A	Abdominal surgery	morphine or fentanyl	0.45	41	70	27	n/a	increase
Zhang et al 2013	128	A	Gastrectomy	fentanyl	0.37	54	53	21	n/a	no effect
Chou et al 2006b	120	A	Total knee arthroplasty	morphine	0.49	62	27	11	no effect	increase
Thomazeau et al 2016	109	M	Knee replacement	morphine	0.08	90	16	1	no effect	no effect
Fukuda et al 2010	108	A	Mandibular osteotomy	fentanyl	0.46	31	54	23	n/a	increase
Kolesnikov et al 2011	102	C	Radical prostatectomy and hysterectomy	morphine	0.11	82	17	3	no effect	no effect
Janicki et al 2006	101	C	Laparoscopic surgery	morphine	0.16	70	30	1	no effect	no effect
Lee et al 2016	88	A	Tonsillectomy	morphine	0.28	66	38	14	increase	no effect
De Gregori et al 2013	98	C	Major abdominal surgery	morphine	0.18	67	26	5	n/a	no effect
Chou et al 2006a	80	A	Hysterectomy	morphine	0.34	43	19	18	no effect	increase
Cobault et al 2006	74	M	Colorectal surgery	morphine	0.04	57	1	2	n/a	no effect
Henker et al 2012	68	C	Orthopaedic surgery of upper or lower limb	fentanyl, hydromorphone, morphine, meperidine	0.14	51	15	2	increase	no effect
Bruehl et al 2006	48	M	Coronary artery bypass	Morphine, oxycodone	0.13	37	10	1	n/a	no effect

Table 4. The associations studies of OPRM1 rs1799971

***OPRM1* rs1799971**

	Phenotype	Association	Reference
Pain			
	Postoperative pain	Negative	Walter 2009
	Experimental pain	Inconclusive	Fillingim 2005
	Cancer pain	Negative	Walter 2009
	Chronic pain	Negative	Janicki 2006
Opioids			
	Postoperative opioid consumption		See Table 3.
	Postoperative nausea and vomiting	Negative	Chou 2006a, Zhang 2011, Sugino 2014
	Severe outcome after drug overdose	Positive	Manini 2013
	Opioid dependence	Positive	Haerian 2013, Kumar 2012
Addiction			
	Substance dependence	Positive	Schwantes-An 2016
	Food addiction	No	Davis 2014
	Alcohol induced euphoria	Positive	Ray 2004, Berettini 2016
	Alcohol addiction	Positive / No	Kumar 2012, Rouvinen-Lagerström 2013
	Tobacco smoking	Inconclusive	Frances 2015, Verhagen 2012, Wang 2015
	Naltrexone effect: relapse rate	Negative	Chamorro 2012
	Naltrexone effect: abstinence	No	Chamorro 2012
Somatic health			
	Breast cancer incidence	Positive	Cieslinska 2015
	Breast cancer mortality	Negative	Bortsov 2012
	Breast cancer stage at diagnosis	Negative	Bortsov 2012
	HIV infection severity	Positive	Proudnikov 2012
	HIV treatment efficacy	Negative	Proudnikov 2012
	Epilepsy (IGE and IAE)	No / Positive	Barratt 2006, Sander 2000
	Pruritus in primary biliary cirrhosis	Negative	Wei 2008
	Migraine pain severity	Positive	Menon 2012
Mental health			
	Schizophrenia	Negative / Positive	Ding 2013, Sery 2010
	Suicidal thoughts	No	Arias 2011
Other			
	Speed dating success (women / men)	Positive / Negative	Wu 2016
	Reinforcement learning	Negative	Lee 2011
	Venlafaxine effectivity on anxiety disorders	No	Cooper 2013
	Preference for high fat, high sugar foods	Positive	Davis 2011
	Binge eating disorder	Positive	Davis 2009
	Obesity	Negative	Davis 2009

2.3.2.2 *FAAH* and the endocannabinoid system

The endocannabinoid system consists of the endocannabinoid receptors located in various parts of the CNS, peripheral tissues and their endogenous ligands. The most expressed receptors are the CB_1 and CB_2 receptors (Lu et al., 2016), which are both G protein coupled receptors that inhibit the intracellular cAMP cascade. CB_1 receptors are highly expressed in the CNS, particularly in the cortex, basal ganglia, hippocampus, and cerebellum. They usually locate at the axon terminal or in its close proximity, but not in the active zone. CB_2 receptors are less abundant in the neurons and locate mainly on immune-modulating cells, including microglia in the brain. The hypothesis is that they act as immunomodulatory agents. In addition, CB_2 receptors might also play a role in pathological processes such as nerve injury. Endocannabinoids also activate other receptors such as peroxisome proliferator activated receptors (PPARs) and TRP channels (Lu et al., 2016).

Endocannabinoids comprise of a common backbone structure that consists of 19 carbon atoms and an individual polar head group (Freund et al., 2003) (Figure 4). The most studied and therefore best-known endocannabinoids are N-arachidonoyl ethanolamide (AEA; anandamide) and 2-arachidonoyl glycerol (2-AG). Both endocannabinoids are produced on demand by one or two rapid enzyme reactions from precursors in the cellular lipid membrane, and are then released straight to the extracellular space. 2-AG acts as a strong agonist for both CB_1 and CB_2 receptors, while anandamide is a weak agonist for CB_1 and an extremely weak agonist for the CB_2 receptor. Activation of CB_1 or CB_2 receptors exerts diverse consequences on cellular physiology, including synaptic function, gene transcription and cell motility (Lu et al., 2016).

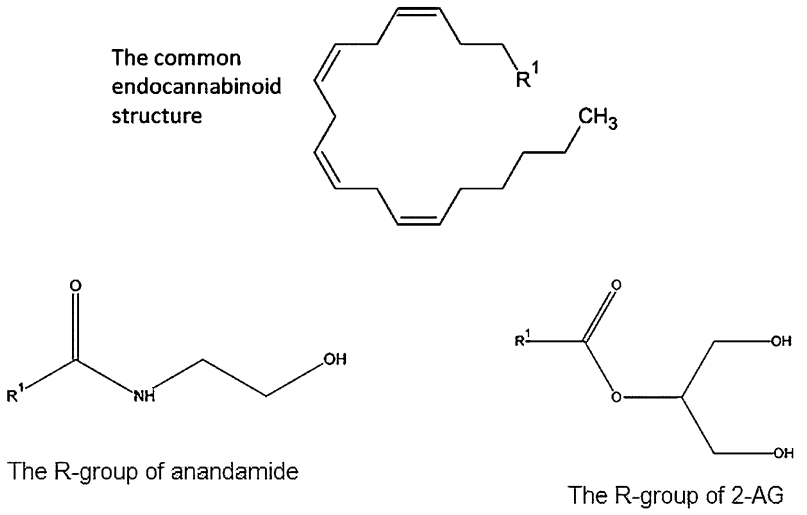

Figure 4. The common 19-coal structure of endocannabinoids and the R-groups of anandamide and 2-AG.

After release to the synaptic cleft, anandamide is taken up by a transporter on the glial cells and is then hydrolysed by FAAH to arachidonic acid and ethanolamine (Figure 5). Another metabolic pathway for anandamide goes through cyclo-oxygenase 2 (COX-2) and yields prostamides. 2-AG is degraded primarily by monoacylglycerol lipase alpha/beta domain-containing hydrolase 6 and alpha/beta domain-containing hydrolase 12 (Figure 7). Additional minor metabolic pathways include oxidation by COX-2 and hydrolysis by FAAH (Lu et al., 2016).

FAAH is capable of hydrolysing many fatty acids. The inhibition of FAAH raises concentrations of ethanolamides, such as anandamide, palmityol and oleonyl amides, which act on both CB_1 receptors and a multitude of other receptors outside the endocannabinoid system. This has made FAAH an interesting target for pharmacological interventions and several studies have investigated the association between FAAH and clinical conditions such as obesity, addiction, neurodegenerative diseases and inflammatory diseases (Alhouayek et al., 2012; Engeli 2008; Micale et al., 2007),

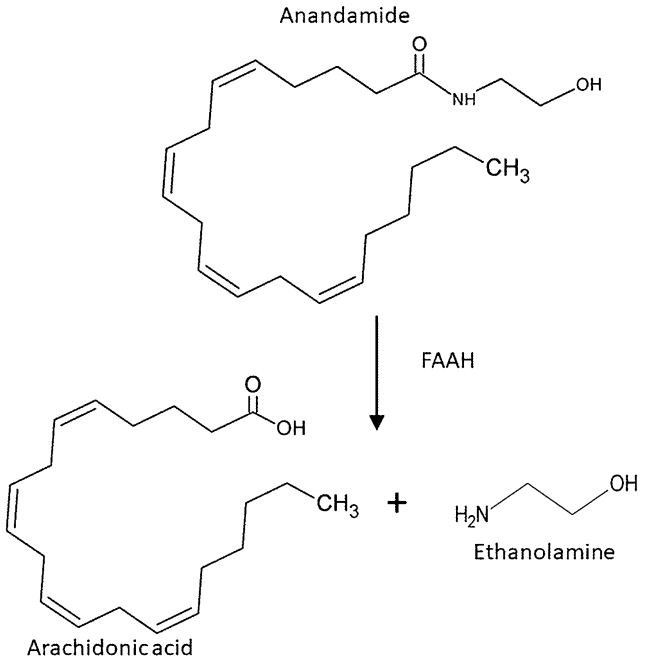

Figure 5. The main metabolic pathway of anandamide by FAAH to arachidonic acid and ethanolamine.

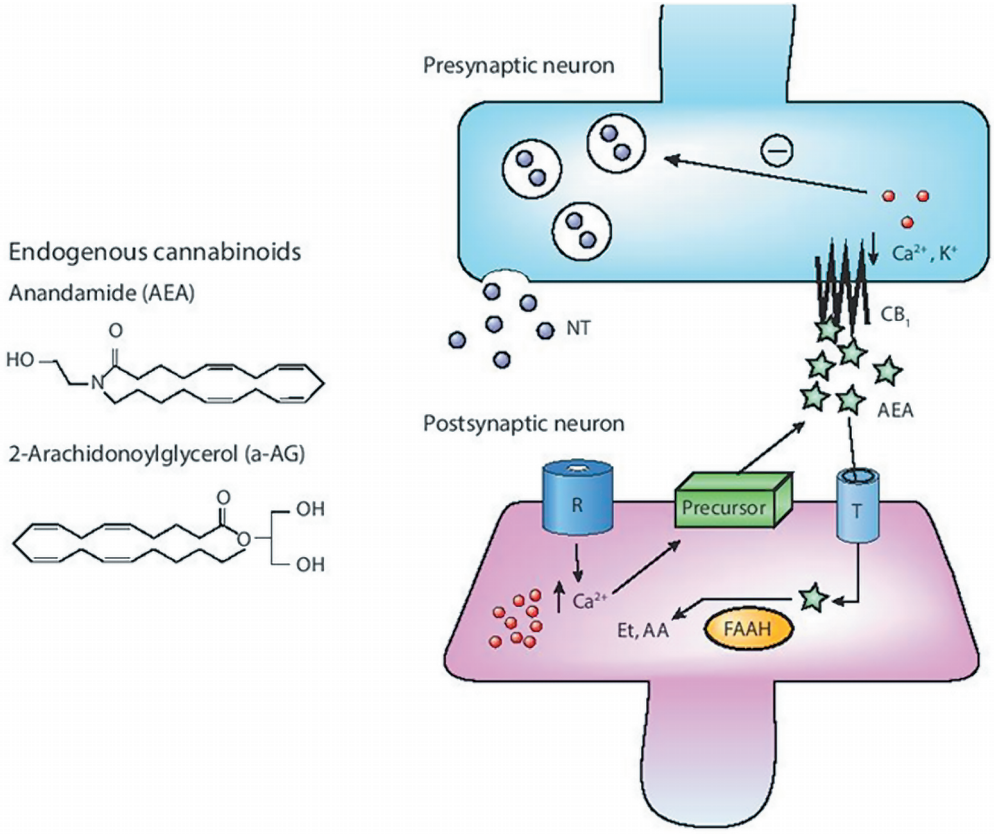

Figure 6. Encocannabinoid activity in the synapse. After the activation of the postsynaptic neuron AEA is released to the synaptic cleft where it activates CB1 receptor inhibiting the release of neurotransmitters. AEA is removed from the synaptic cleft by a unknown transporter and the hydrolysed by FAAH: NT=neurotransmitter, R=receptor, AEA=anandamide, CB1=cannabinoid receptor 1, T=transporter, FAAH=fatty acid amide hydroxylase, Et=ethanolamine, AA=arachidonic acid

The gene coding for FAAH, located in the short arm of chromosome 1, comprises of almost 20,000 base pairs and contains 15 exons. *FAAH* is expressed in tissues throughout the body, with the highest levels of expression occurring in the prostate, testis, thyroid, small intestine, colon and kidney. There are currently over 1,700 known variants in *FAAH*, of which, only 48 have a minor allele frequency above 5%. Of these, 42 are single nucleotide variants, four deletions and two insertions (NCBI Gebe Database: *FAAH*). The most interesting is a single nucleotide polymorphism rs324420 that causes a missense mutation changing proline to threonine in the surface loop on the cytoplasmic face of FAAH that reduces the expression of FAAH in human T-lymphocytes by 50%. So far, the change in expression seems to be as a result of post-translational modulations, possibly due to increased susceptibility to degradation by cellular proteases (Chiang et al., 2004). The *FAAH* rs324420 has been extensively studied and has been associated with multiple phenotypes (Table 5). The

other 47 common SNPs locate either up- or downstream from the coding region or in introns.

With regard to pain, *FAAH* -/- mice had a 50 to 100-fold decrease in hydrolysis of anandamide in their CNS. The mice exhibited reduced pain sensitivity, which could be reversed by a CB_1 antagonist (Cravatt et al., 2001). Numerous FAAH inhibitors have been synthesized and in animal tests, they have shown promising results (Ahn et al., 2011; Li et al., 2012; Lichtman et al., 2004). One FAAH inhibitor BIA 10-2474 was even subject to a phase I clinical trial on humans, although the trial had to be halted due to unexpected adverse events (Mallet et al., 2016).

One previous study has addressed *FAAH* polymorphisms and pain. This study showed association between three *FAAH* SNPs (rs932816, rs4141964 and rs2295633) and cold pain sensitivity. Rs4141964 was also shown to reduce cold withdrawal time by 15% (Kim et al., 2006).

Table 5. The association studies of *FAAH* rs324420

***FAAH* rs324420**

	Phenotype	Association	Author
Pain			
	Cold pain	No	Kim 2006
Addiction			
	Problem drug use	Positive	Flanagan 2006, Sipe 2002
	Cannabis use disorder	Positive / Negative	Melroy-Greif 2016, Tyndale 2007
	Marijuana craving	Negative	Haughey 2008
	Happiness after marijuana smoking	Negative	Schacht 2009
	Marijuana withdrawal symptom severity	Negative	Schacht 2009
	Regular use of alcohol	No	Tyndale 2007
	Risky alcohol consumption	Negative	Buhler 2014
	Alcohol dependence	No	Tyndale 2007, Iwasaki 2007
	Regular use of nicotine	No	Tyndale 2007
	Nicotine dependence	No	Tyndale 2007
	Regular sedative use	Negative	Tyndale 2007
	Sedative dependence	No	Tyndale 2007
	Heroin addiction	No	Proudnikov 2010
	Methamphetamine dependence	Positive / no	Sim 2013, Morita 2005
	Arousal after amphetamine use	Positive	Dlugos 2010
	Regular sedative use	Positive	Tyndale 2007
	Regular stimulant use	No	Tyndale 2007
Opioid system			
	Opioid induced respiratory depression	Positive	Chidambaran 2017, Sadhasivam 2015
	Opioid induced nausea and vomiting	Positive	Chidambaran 2017, Sadhasivam 2015

***FAAH* rs324420**

	Phenotype	Association	Author
CNS effects			
	Threat related amygdala reactivity	Negative	Hariri 2009
	Reward-related ventral striatal reactivity	Positive	Hariri 2009
	Fronto-amygdala connection	Positive	Dincheva 2015
Mental health			
	Fear extinction	Positive	Dincheva 2015
	Anxiety level (STAI)	Negative	Dincheva 2015
	Anxiety response to stress	Negative	Spagnolo 2016
	PTSD symptoms severity	Negative	Spagnolo 2016
	Emotional-motivational reactivity	Negative	Conzelmann 2012
	Anorexia nervosa	Positive	Monteleone 2009
	Bulimia nervosa	Positive	Monteleone 2009
	Binge eating disorder	No	Monteleone 2008
	Antisocial personality disorder	Positive	Hoenicka 2007
	Schizophrenia	No	Morita 2005
	Bipolar disorder	No	Piscanu 2013
Body composition and related metabolic effects			
	Obesity	Positive (2) / no (4)	Sipe 2005, Monteleone 2008, Jensen 2007, Papazoglou 2008, Lieb 2009, Muller 2010
	Hypocaloric diet's effect on insulin levels	Negative	de Luis 2013
	Prevalence of metabolic syndrome	No	de Luis 2012
	Antipsychotic drug associated weight gain	No / Positive	Nurmi 2013, Monteleone 2010
	Decrease in cholesterol and triglycerides after high polyunsaturated fat diet	Positive	Aberle 2007, de Luis 2013
	High BMI	Positive	Zhang 2009, de Luis 2010
	Increased triglycerides	Positive / Negative	Zhang 2009, de Luis 2010
	Level of HDL cholesterol	Negative	Zhang 2009
	Insulin sensitivity	No	Zhang 2009
	Waist circumference	No	De Luis 2010
	Insulin level	Positive	De Luis 2012
Somatic health			
	Myocardial infarction	Positive	Chmelikova 2015
	Irritable bowel syndrome	No	Jiang 2014
	Functional gastrointestinal disorders	Positive	Camilleri 2008
	Crohn's disease symptom severity	Positive	Storr 2009
	Earlier onset of ulcerative colitis	Positive	Storr 2009
	Systolic blood pressure in young males	Negative	Sarzani 2008
	Heart rate after marijuana smoking	Positive	Schacht 2008
	Risk for acute respiratory distress syndrome	Positive	Tejera 2012
Pharmacological			
	Placebo effect	Positive	Pecina 2014
	Citalopram treatment efficacy	No	Mitjans 2012
	Lithium response	No effect	Pisanu 2013

2.3.2.3 CYP3A

Cytochrome P-450 3A is probably the most pharmacokinetically important enzyme in the metabolism of drugs as this protein participates in the metabolism of most of the drugs in clinical use (Burk et al., 2004). CYP3A and its isoenzymes CYP 3A4, 3A5 and 3A7 are highly expressed in the small intestine and the liver. Lower rates of expression can be found in several other tissues, but the clinical significance of this remains uncertain.

CYP3A isoenzyme genes are all located next to each other in the long arm of chromosome 7 and around 60 known polymorphisms have been identified in the gene (NCBI Gene Database: CYP3A4). Most of them are SNPs and have very low allelic frequencies, which makes their effect hard to study. Of the 60, only eight polymorphisms have shown to alter CYP3A4 function in vitro and two in vivo (Table 6).

Investigations of *CYP3A4**18A (rs28371759) by observing the concentration of midazolam have seemed to demonstrate an increase in the *CYP3A4* activity when tested in vitro but a decreased activity when tested in vivo (Kang et al., 2009). *CYP3A4**22 (rs35599367) has been deemed the most clinically relevant polymorphism in the *CYP3A4* gene as this polymorphism has been shown to reduce both *CYP3A4* hepatic expression and enzyme activity by reducing the production of a functional full-length mRNA (Wang et al., 2011). *CYP3A4**22 locates in an intron but seems to alter the splicing pattern of the mRNA (Wang et al., 2016). *CYP3A4**22 carriers metabolize many drugs slower than wild-type individuals do and clinical differences have been shown with statins, cyclosporine, midazolam and erythromycin (Wang et al., 2011; Elens et al., 2013). CYP3A4 enzyme activity can also be easily upregulated by many substrates, which can be endogenous, like corticosteroids or xenobiotics, such as phenytoin or rifampicin.

CYP3A5 is expressed in the small intestine, liver, kidney and many other organs (NCBI Gene Database: CYP3A5). The relative amount of CYP3A5 in the total CYP3A pool varies greatly between individuals (Burk et al., 2004) and so far, the most influential polymorphism has been found to affect *CYP3A5* expression is 6986A>G (rs776746, *CYP3A5**3). This polymorphism results in a cryptic splice site in the intron 3 of the *CYP3A5* leading to premature termination of transcription and a shortened mRNA. The transcription product is usually quickly degraded (Kuehl et al., 2001). Nevertheless, some pre-mRNA molecules escape the altered splicing effect resulting in completely normal, functional mRNAs (Lin et al., 2002) meaning that even individuals homozygous for the G-allele express some amount of *CYP3A5*.

The individual effect of *CYP3A5* polymorphism on drug metabolism is hard to study since the enzyme is always co-expressed with CYP3A4. Often both enzymes participate in the same metabolism and are highly similar with respect to activities, substrates and metabolic products. Therefore, many studies have found completely different effects concerning *CYP3A4* polymorphisms and drug effects (Burk et al., 2004).

The effect of *CYP3A4*/5 activity on oxycodone metabolism has been researched primarily by the co-administration of oxycodone together with a known CYP3A4/5 inducer or inhibitor. Strong CYP3A4 inhibitors have been shown to increase oxycodone exposure by 200-300% (Hagelberg et al., 2009; Nieminen et al., 2010b). In contrast, when used together with a CYP3A4 inducer the oxycodone exposure decreased by 50-85% (Nieminen et al., 2009; Nieminen et al., 2010a). With regard to the effect of *CYP3A4*/5 polymorphism on oxycodone metabolism, to date no studies have been performed, however, fentanyl has been studied concerning the effect of *CYP3A4**18. Of the three studies of this polymorphism and post-operative fentanyl consumption, two found no significant change in the dose of fentanyl required for analgesia (Tan et al., 2012; Kim et al., 2013). The other study however, did notice a statistically significant difference in fentanyl requirement between *CYP3A4**18 homozygotes and wild-type homozygotes at 48h after the operation but no difference between heterozygotes or either homozygotes (Liao et al., 2013). One study has looked at *CYP3A4**22's effect to fentanyl plasma concentrations but combined this to multiple other phenotypes (Barratt et al., 2014) and consequently, no conclusions about the individual effect of the polymorphism can be drawn.

Table 6. The polymorphism shown to affect CYP3A4 activity in vitro or in vivo. Altered from *CYP3A4* Allele Nomenclature (CYP3A4 Allele Nomenclature, 2017)

Allele	Nucleotide changes	Enzyme activity		References
		In vivo	In vitro	
*CYP3A4*8*	13908G>A		Decr	Eiselt et al, 2001
*CYP3A4*11*	21867C>T		Decr	Eiselt et al, 2001 Murayama et al., 2002
*CYP3A4*12*	21896C>T		Decr	Eiselt et al, 2001
*CYP3A4*13*	22026C>T		Decr	Eiselt et al, 2001
*CYP3A4*16A*	15603C>G		Decr	Lamba et al, 2002 Murayama et al., 2002
*CYP3A4*16B*	15603C>G; 20230G>A		Decr	Fukushima-Uesaka et al, 2004 Murayama et al., 2002
*CYP3A4*17*	15615T>C		Decr	Dai et al, 2001
*CYP3A4*18A*	20070T>C	Decr	Incr	Dai et al, 2001 Kang et al., 2009
*CYP3A4*22*	15389C>T	Decr.		Wang et al., 2011 Elens et al., 2011a Elens et al., 2011b

2.3.2.4 CYP2D6

CYP2D6 belongs to the same cytochrome P450 family as the CYP3A enzymes. *CYP2D6* locates in chromosome 22 and consists of nine exons and eight introns (NCBI Gene Database: CYP2D6). The gene possesses much variation with multiple allelic

variants and at least 22 null-alleles, known to result in a non-functional protein (Zhou 2009b). Therefore, there is a high variation in enzyme activity between individuals, which ranges from a complete lack of enzyme activity to ultra-rapid activity caused by copy number variants. Traditionally individuals have been divided into 4 groups based on their gene composition. *Poor metabolizers (PM)* possess two null-alleles resulting in complete lack of a functioning CYP2D6 enzyme. *Intermediate metabolizers (IM)* have a severely impaired but somewhat functional version of the enzyme and usually these individuals carry multiple alleles that decrease the enzyme activity. *Extensive metabolizers (EM)* have a normally functioning gene and these individuals compose the majority of the Caucasian population. In *ultra-rapid metabolizers (UM),* there is multiple copies of the gene, resulting in a highly increased expression of the enzyme and enzyme activity. Recently, the classical 4-step classification has been replaced with a continuous variable: the CYP2D6 activity score. The activity score predicts the CYP2D6 activity with more precision and is more easily interpreted and modified to meet special needs (Gaedigk et al., 2008). In the activity score method, each polymorphism is assigned a value that represents the polymorphisms effect on enzyme activity. The activity score is the calculated by summing up the values of the individual's alleles. The values given to each polymorphism are listed in Table 7.

Table 7. Values assigned to *CYP2D6* alleles in the activity score method. Each allele is given a number representing its effect on the enzymes activity. Then the values of the two alleles are summed to form the activity score of the pair. *N* represents the number of duplication polymorhisms. Modified from the original article by Gaedick et al. (Gaedigk et al., 2008).

Value assigned to allele	Alleles
0	**3*, **4*, **4xN*, **5*, **6*, **7*, **16*, **36*, **40*, **42*, **56B*
0.5	**9*, **10*, **17*, **29*, **41*, **45*, **46*
1	**1*, **2*, **35*, **43*, **45xN*
2	**1xN*, **2xN*, **35xN*

Since CYP2D6 is responsible for the metabolism of many clinically used drugs such as antiarrhythmics, selective serotonin reuptake inhibitors, opioids, neuroleptics and anticancer agents, the high variation in enzyme activity causes problems that possibly expose the patient to life-threatening adverse effects. As a result, CYP2D6 has been extensively investigated and Zhou et al. (Zhou 2009a; Zhou 2009b) have compiled the results of *CYP2D6* polymorphism effects on different drugs in two extensive publications.

With regard to oxycodone analgesia, CYP2D6 metabolism seems to have no clinically significant effect. Although it has been suggested that oxymorphone contributes to oxycodone analgesia, the amount of oxymorphone produced by CYP2D6 remains too low for any significant effect (Lemberg et al., 2009). The only study on post-operative analgesia found no significant association between *CYP2D6* genotype and post-operative oxycodone requirements (Zwisler et al., 2010).

3. AIMS OF THE STUDY

The main purpose of this study was to identify factors affecting post-operative analgesia that could help anaesthesiologists and surgeons to recognise patients at risk of severe post-operative pain and high requirement of opioid analgesics, oxycodone as an example.

The specific aims of this thesis were:

I. To define specific patient-dependent (demographic, clinical), genetic (*OPRM1, FAAH, CYP3A4/5* and *CYP2D6*)) and surgery-dependent factors affecting post-operative oxycodone requirements and analgesic oxycodone concentrations after breast cancer surgery and to estimate their effects.

II. To define specific patient-dependent (demographic, clinical), genetic (*OPRM1* and *FAAH*) and surgery-dependent factors affecting post-operative pain intensity after breast cancer surgery and to estimate their effects.

III. To define specific patient-dependent (demographic, clinical) and genetic (*OPRM1* and *FAAH*)) factors affecting experimental pain sensitivity and whether these factors associate with post-operative pain intensity.

4. MATERIALS AND METHODS

4.1 Ethical considerations

The study protocol was approved by the coordinating ethics committee (136/E0/2006) and the ethics committee of the Department of Surgery (Dnro 148/E0/05) of the hospital district of Helsinki and Uusimaa prior to the commencement of the study. All patients volunteered for the study and were informed of both the study procedure and any possible risks. Each patient provided a written informed consent, which was collected and archived by the research nurse or physician. The patients were free to withdraw from the study at any time.

4.2 Study subjects

The patients were recruited from the Breast Surgery Unit of the Helsinki University Central Hospital between August 2006 and December 2010. The inclusion criteria comprised of the following: a female patient diagnosed with unilateral, non-metastasized breast cancer, aged 18-75 years. All patients were to undergo either breast conserving surgery (BCS) or mastectomy with sentinel node biopsy or axillary clearance. Exclusion criteria included metastasized cancer (other than axillary lymph nodes), clinically significant liver or kidney failure, previous breast cancer surgery on the same side, BMI >35 (from the patient number 100 on), alcoholism, contraindications for use of oxycodone, and severe psychiatric disease. Additionally, patients undergoing immediate breast reconstruction were also excluded from the study. The type of surgery used was decided based on the characteristics of the tumour and according to the patient's wishes.

During the recruitment period, there were 1,536 eligible patients of whom 387 were excluded due to logistic reasons. Of the 1,149 patients invited, 126 declined and 23 were withdrawn due to contraindications to the anaesthetic protocol, change in the type of surgery, violation of the study protocol or due logistic reasons. The final cohort consisted of 1,000 women (Figure 7).

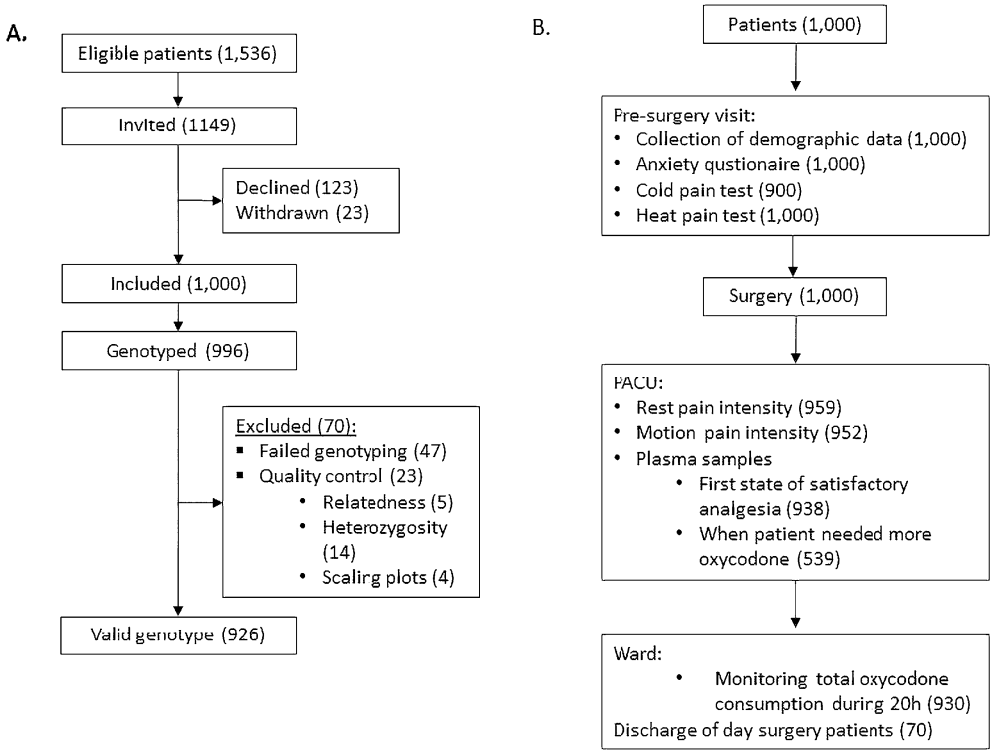

Figure 7. Flowcharts of A. Patient selection and genotyping and B. Study procedures.

4.3 Study protocol

The day prior to surgery, each patient met with the research nurse in order to collect her background data. This included medical information and demographic background data: age, weight and height, diagnoses, medications, number of previous operations (other than breast surgery), previous chronic pain of any kind, preoperative use of hormonal replacement therapy, use of alcohol (no/less than six doses per week/more than six doses per week), alcohol problems in the family (no/yes) and smoking (never/yes/stopped). Since all study patients were quite healthy (ASA grade 1 or 2), the patients were not automatically subjected to laboratory testing before surgery. Thus, no consistent data of liver or kidney function at the time of the operation were available.

The patients were asked to assess the preoperative pain in the area to be operated on using a numerical rating scale 0-10 (NRS 0-10) zero indicating no pain and 10 indicating the worst pain imaginable. Finally, all patients filled in psychological questionnaires to assess levels of depressive mood and anxiety. The questionnaires used included the Becks Depression Inventory (BDI) (Beck et al., 1961) and Spielberger State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983). STAI consist of 20 questions about anxiety which are given a 4-point frequency scale.

Patient can estimate, how often he/she experiences these feelings. Minimum score is 20 correlating to low anxiety, and maximum score 80 correlating to high anxiety. BDI consist of 21 questions with four answer oprtions. Patient chooses which one best descipes his/her state. Answers are rated on a 0-3 point scale. Total score of 0-9 indicates minimal depression, 10-18 mild depression, 12-29 moderate depression and 30-63 severe depression.

4.3.1 Experimental pain test

On the day before surgery, patients underwent two experimental pain tests for heat and cold pain. A careful explanation of these tests was performed with every patient beforehand.

Heat pain assessment was carried out using the 16 mm x 16 mm thermode of the TSA-II NeuroSensory Analyzer (Medoc Ltd., Ramat Yishai, Israel) by placing it on the patient's hand's volar side contralateral to the surgery. In the first step, the thermode was heated to 43°c for 5 seconds and the patient was asked to report the unpleasantness of the sensation using NRS 0-10. In the next step, the thermode was heated to 48°C and placed on the same area for 5 seconds. Again, the patient was asked to assess the unpleasantness of the sensation using NRS 0-10.

In the cold pain test the patient was asked to immerse her hand into a cold water bath at between 2-4°C (JULABO USA Inc., Allentown, PA, USA) and keep it there for the maximum time that could be tolerated with a cut-off time of 90 seconds. Pain intensity and unpleasantness was recorded every 15 seconds using an NRS 0-10 and the NRS score was also recorded at the time of hand withdrawal. For the first 100 patients this cold pain test was not conducted due to the unavailability of the cold-water bath device.

4.3.2 Anaesthesia

The anaesthetic procedures were standardised - patients were pre-medicated with diazepam 2.5 – 15 mg and 1g acetaminophen. In the operating theatre, an intravenous (i.v.) infusion of remifentanil (0.05-0.25 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was started. Anaesthesia was induced and maintained with i.v. propofol (2-3 $mg \cdot kg^{-1}$ and 50-100 $\mu g \cdot kg^{-1} \cdot min^{-1}$). Rocuronium was administered to achieve sufficient muscle relaxation for the tracheal intubation and to keep the train-of-four at 0-10% (E-NMT: General Electrics Healthcare Finland, Helsinki, Finland). Mechanical ventilation was adjusted with a 1:1 mixture of oxygen and nitrous oxide to keep normocapnia. During the closure of the skin, the remifentanil infusion was stopped, and patients were given i.v. fentanyl (0.1 $\mu g \cdot kg^{-1}$), i.v. ondansetron 4mg and i.v. droperidol (0.01 $mg \cdot kg^{-1}$). Before moving the patient to the post-anaesthesia care unit (PACU), the neuromuscular block was reversed using i.v. neostigmine 2.5mg

and i.v. glycopyrrolate 0.5mg. At this point, the intubation tube was removed and a blood sample for DNA analyses was drawn during the anaesthesia to minimize the need for punctures.

4.3.3 Postoperative pain assessment and administration of oxycodone

In the PACU, patients were asked to assess their pain intensity during rest and motion using NRS 0-10. For the motion pain assessment, the patient was asked to raise the arm ipsilateral to surgery up to 90 degrees. The patient was then titrated to satisfactory anaesthesia (NRS 0-10 < 4) by the research nurse who asked about the pain intensity every 5 min and administered 1-3mg i.v. oxycodone accordingly. Once the patient reported NRS < 4 or indicated otherwise satisfaction with the anaesthesia, a blood sample was taken for the plasma oxycodone concentration measurement. The research nurse continued monitoring the anaesthesia by asking the patient to rate pain intensity every 15 min. When the patient needed a new dose of oxycodone, a second blood specimen was collected for the oxycodone plasma concentration measurement prior to the administration of a new oxycodone dose.

Analgesic concentration in this study refers to the plasma concentration at which the patient felt that the effect of the drug was satisfactory (provided adequate analgesia). No therapeutic range or analgesic concentration for oxycodone or other opioids has been specified.

After approximately 2 hours in the PACU, patients were provided with patient-controlled analgesia devices (PCA) (Abbott Laboratories, North Chicago, IL, or CADD-LegacyT: Deltec, Inc., St. Paul, MN), the research nurse explained their use and the patients were transferred to the ward. PCA oxycodone was available for up to 20h except for the 70 day-surgery patients who were discharged during the operation day. The patient self-administered i.v. oxycodone from the PCA in 1 -3.5 mg boluses with an 8 - 10 min lock-out time. The total oxycodone consumption and any adverse effects during the first 20 post-operative hours were recorded. All patients were also provided with 1g of paracetamol as basic analgesic every 8 hours.

4.4 SNP selection

Originally eight genes (*OPRD1*, *OPRM1*, *FAAH*, *ABCB1*, *TRPA1*, *TRPV1*, *GCH1*, *COMT*) were selected as primary candidate genes for pain perception and relief on the basis of the findings from previous studies (Kim et al., 2006; Campa et al., 2008; Diatchenko et al., 2005; Tegeder et al., 2006). One or several functional or reported candidate SNPs was/were selected from each of these prioritized genes and multiplexed in order to be genotyped by Sequenom MassArray. Altogether, 22 SNPs were genotyped. This Sequenom-generated dataset also contained the *OPRM1*

rs1799971 SNP and two SNPs (rs3766246 and rs4141964) located within the *FAAH* gene. The *OPRM1* and *FAAH* genes were selected for a more thorough investigation because of the promising results with these two SNPs in preliminary analyses and results from previous studies.

In study I addressing oxycodone and metabolite concentrations, one SNP from CYP3A4 and CYP3A5 were included in the analysis. CYP3A4/5 is the main metabolizer of oxycodone. The two SNPs *CYP3A4* rs35599367 (*22) and *CYP3A5* rs776746 (*3) were selected since both have been shown to affect the pharmacokinetics and drug response of many drugs (Wang et al. 2011; Elens et al. 2011 a & b; Jacobson et al., 2011).

4.5 Genotyping

DNA was extracted from the peripheral blood of 996 patients using the Autopure LS automated DNA purification instrument (Gentra Systems, Inc., Minneapolis, MN, USA).

Genotyping of the 22 candidate SNPs was performed at the Institute for Molecular Medicine Finland FIMM Technology Centre at the University of Helsinki (Helsinki, Finland). The SNPs were genotyped using the MassARRAYsystem and iPLEX Gold Single Base Extension chemistry (Sequenom, San Diego, CA, USA). Both duplicate and positive-negative control samples were included in each plate in order to confirm the accuracy of the genotyping results. This set included *OPRM1* rs1799971 and *FAAH* rs3766246 and rs4141964.

Patients were genotyped for 10 *CYP2D6* SNPs and copy number variation using Taqman genotyping assays and a copy number assay targeting exon 9 (Pietarinen et al., 2016). *CYP2D6* metabolizer groups were inferred from the genotype data using the activity score method (Gaedigk et al., 2008). Both *CYP3A4* rs35599367 (*22) and *CYP3A5* rs776746 were genotyped using Taqman genotyping assay (ThermoFisher Scientific, Waltham, MA, USA), *CYP3A4* rs35599367 (*22) with a commercially available kit (C_59013445_10) and *CYP3A5* rs776746 (*3) with a custom genotyping assay.

The GWA genotype data were produced at the Wellcome Trust Sanger Institute (Hinxton, UK) on the Human OmniExpress Illumina BeadChip (Illumina, Inc., San Diego, CA, USA) and were blind to phenotypic information. Of the 996 samples, genotyping of 47 failed due to the low quality of the DNA or other technical issues. The quality control procedure of the remaining 949 samples consisted of checking the data for heterozygosity (14 fails), unexpected relatedness (5 fails) and creating multidimensional scaling plots (4 genetic outliers rejected based on dimensions 9-10). The SNPs were filtered based on minor allele frequency (MAF >0.005), Hardy-Weinberg equilibrium (HWE p >$1x10^{-6}$) and success rate (>0.97). After these

exclusions, the final cohort consisted of 926 individuals with a mean genotyping success rate of 0.997 (Figure 7).

OPRM1 rs1799971 genotype data was extracted from candidate SNP data genotyped using the MassArray platform. The other 20 *OPRM1* SPNs were extracted from the GWAS data located within the surrounding 20 kb area of the gene (chr6: 154380 – 154476 kb, NCBI36/hg18).

In Study II that focused on *FAAH*, the genotype data was extracted from the GWA data. In the GWA dataset, eight SNPs were located within the *FAAH* gene or the surrounding 20 kb area (chr1: 46,612 – 46,672 kb, NCBI36/hg18). Prior to the availability of the GWA data, two candidate SNPs (rs4141964 and rs3766246) were genotyped with the MassArray platform. As a result, SNP rs3766246 was available in both the candidate SNP and GWA data.

4.6 Measurement of oxycodone and its metabolites

Plasma concentrations of oxycodone and three of its metabolites were quantified from 0.5 ml plasma samples using an API 3000 liquid chromatography-tandem mass-spectrometry system (Sciex Division of MDS, Toronto, Ontario, Canada) as described previously (Neuvonen M et al., 2008). The method was validated according to the Food and Drug Administration guidelines. (FDA Guidance, CDER, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research and Center for Veterinary Medicine (CVM): Guidance for the Industry, Bioanalytical Method Validation, May 2001). The lower limits of quantification for oxycodone and oxymorphone and noroxycodone and noroxymorphone were 0.1 ng/mL and 0.25 ng/mL, respectively. The inter-day coefficient of variation for all analytes at the relevant concentrations was below 15%. Oxycodone and its metabolite concentrations below the limit of quantification were replaced with half of the quantification limit, except for those patients who did not need any oxycodone and were thus excluded from the respective analyses. Oxycodone and its metabolite concentrations were logarithmically transformed before statistical analysis.

4.7 Statistical analyses

4.7.1 Power calculations

Power calculations for *OPRM1* rs1799971 and *FAAH* SNPs were conducted using the freely available Quanto software, version 1.2.4 (University of Southern California 2009). This software was used to estimate the effect sizes that the polymorphisms should have on the studied phenotypes of our study sample in

order to have sufficient power to detect the effect. The range of allele frequencies in *FAAH* SNPs was considered by performing power calculations for the most and least frequent SNPs.

4.7.2 Defining factors affecting postoperative motion pain intensity, oxycodone dose required for satisfactory analgesia and oxycodone concentration at satisfactory analgesia.

In Study I, the possible effects of the variables affecting plasma oxycodone concentrations were investigated using a stepwise, forward linear regression analysis using SPSS Statistics version 22 (IBM, Armonk, NY). The same method was used for the determination of factors associating with experimental cold pain intensity at 30s, post-operative pain intensity and post-operative oxycodone dose requirements. A statistical significance level of $P < 0.05$ for entry into the model and > 0.10 for removal was set. Motion pain intensities, oxycodone doses and oxycodone plasma concentrations did not follow normal distribution, so they were logarithmically transformed before statistical analysis. The factors included in the linear regression for each analysis are given in Table 8 and 9.

Possible effects of *CYP2D6* genotypes on oxycodone metabolite concentrations were investigated with one-way analysis of variance as were the differences between postoperative pain intensity groups. Oxycodone and metabolite concentrations did not follow normal distribution, so they were logarithmically transformed before analysis.

Table 8. Factors tested in the linear regression analysis for cold pain intensity at 30s. BMI =body mass index, NRS = numerical rating scale, STAI = State-Trait Anxiety Inventory

Cold pain intensity at 30s
Age (year)
Weight (kg)
BMI (kg m^{-2})
Smoking (yes/no)
Preoperative chronic pain (yes/no)
Preoperative breast pain (NRS 0-10)
Anxiety (STAI)
OPRM1 rs1799971 genotype
FAAH rs324420 genotype

Table 9. The factors tested in the linear regression analysis for each postoperative primary phenotype. BMI =body mass index, NRS = numerical rating scale, STAI = State-Trait Anxiety Inventory, BCS = breast conserving surgery, SNB = sentinel node biopsy, AC = axillary clearance

Motion pain intensity	**Postoperative oxycodone dose required for satisfactory analgesia**	**Postoperative oxycodone plasma concentration required for satisfactory analgesia**
Age (year)	Age (year)	Age (year)
Weight (kg)	Weight (kg)	Weight (kg)
BMI (kg m-2)	BMI (kg m-2)	BMI (kg m-2)
Smoking (yes/no)	Smoking (yes/no)	Smoking (yes/no)
Preoperative chronic pain (yes/no)	Preoperative chronic pain (yes/no)	Preoperative chronic pain (yes/no)
Preoperative breast pain (NRS 0-10)	Preoperative breast pain (NRS 0-10)	Preoperative breast pain (NRS 0-10)
Anxiety (STAI)	Anxiety (STAI)	Anxiety (STAI)
Cold pain intensity at 30s (NRS 0-10)	Cold pain intensity at 30s (NRS 0-10)	Cold pain intensity at 30s (NRS 0-10)
The total time patients kept their hand immersed in cold water (s)	The total time patients kept their hand immersed in cold water (s)	The total time patients kept their hand immersed in cold water (s)
Heat pain intensity (NRS 0-10)	Heat pain intensity (NRS 0-10)	Heat pain intensity (NRS 0-10)
OPRM1 rs1799971 genotype	*OPRM1* rs1799971 genotype	*OPRM1* rs1799971 genotype
FAAH rs324420 genotype	*FAAH* rs324420 genotype	Mastectomy (vr. BCS)
Mastectomy (vs. BCS)	Mastectomy (vs. BCS)	Axillary clearance (vs. SNB)
Axillary clearance (vs. SNB)	Axillary clearance (vs. SNB)	Motion pain (NRS 0-10)
	Motion pain (NRS 0-10)	Resting pain (NRS 0-10)
	Resting pain (NRS 0-10)	*CYP2D6* genoype
	CYP2D6 genoype	*CYP3A4* rs355367 genotype
	CYP3A4 rs355367 genotype	*CYP3A5* rs776746 genotype
	CYP3A5 rs776746 genotype	Lean body weight (kg)

4.7.3 Genetic association studies (studies II and III)

OPRM1 and *FAAH* polymorphisms were assessed for statistically significant deviations from the Hardy-Weinberg equilibrium using the X^2 method with Plink software (Purcell et al., 2007).

Associations between *OPRM1* and *FAAH* with clinical phenotypes were obtained using linear regression. Additive, recessive and dominant models were used to find association with genotype and phenotype data. Since all phenotypic data were not normally distributed, associations were also assessed through permutation testing. Each model was fitted 10.000 times and the original statistics were compared with the test statistics received from permutation.

All association results were corrected using Bonferroni correction to compensate for the increased chance of rare events caused by the multiple testing. The number of tests was set to the number of phenotypes, SNPs and models used. For the 8 *FAAH* SNPs the correction factor was set to 6.2 due to the strong linkage disequilibrium between the SNPs.

For the *FAAH* SNPs haplotype analyses were conducted. Haploblock boundaries and linkage disequilibrium between the SNPs were determined using Haploview software (version 4.2). The haplotype analyses were conducted using Plink software (Purcell et al., 2007).

5. RESULTS

5.1 Characteristics of the patients

The final study cohort consisted of 1,000 women undergoing surgery for breast cancer. The demographics of the patients are presented in Table 10. 420 patients underwent breast conserving surgery with sentinel node biopsy and 206 breast conserving surgery with axillary clearance. Mastectomy was performed on 374 patients, of whom, 140 had sentinel node biopsy and 234 axillary clearance.

Table 10. The means (SD) of demographic factors in the study population. Number of smokers are given as individuals. STAI = State-Trait Anxiety Inventory, NRS = numerical rating scale.

Age (years)	57.0 (9.28)
Height (cm)	165 (5.97)
Weight (kg)	69.1 (12.1)
BMI	25.4 (4.28)
Anxiety score (STAI)	40.3 (11.2)
Preoperative breast pain (NRS)	1.14 (3.41)
Preoperative chronic pain (%)	24.20%
Smoking (never/yes/stopped/periodic)	591/167/228/14

5.2 Genotypes of the patients

With regard to *OPRM1* genotype the study population consisted of 631 AA, 327 AG and 35 GG individuals. The three-genotype groups did not differ significantly for the studied demographics. The *FAAH* genotype frequencies are shown in Table 11 and the combined *OPRM1* and *FAAH* genotypes in Table 12.

Table 11. The genotype counts and minor allele frequencies of the studied *FAAH* polymorphisms. SNP = single nucleotide polymorphism, MM = major allele homozygotes, Mm = heterozygotes, mm = minor allele homozygotes, HWE = Hardy-Weinberg equilibrium.

SNP ID	Nucleotide change	Minor allele frequency	Genotype count			HWE p
			MM	Mm	mm	
rs3863641	A > G	0.38	364	415	144	0.13
rs3766246	G > A	0.38	347	448	128	0.4
rs324420	C > A	0.28	469	380	72	0.75
rs11576941	C > A	0.27	481	373	66	0.62
rs324425	G > A	0.03	865	57	1	1
rs4660928	C > A	0.35	386	422	115	1
rs1571138	G > A	0.28	475	376	72	0.94
rs1535737	G > A	0.35	382	433	108	0.43

Table 12. The counts of pairwise combinations of *OPRM1* rs1799971 and *FAAH* rs324420 genotypes.

rs324420	rs1799971			
	AA	AG	GG	Total
CC	301	158	13	472
AC	234	125	21	380
AA	48	24	0	72
Total	583	307	34	924

Table 13. The genotype counts and minor allele frequencies of CYP3A4 rs355367 and CYP3A5 rs776746

SNP ID	Nucleotide change	Minor allele frequency	Genotype count			HWE p
			MM	Mm	mm	
rs355367	C>T	0.03	885	45	1	0.58
rs776746	G>A	0.08	792	135	3	0.21

5.3 Experimental cold pain

Cold pain intensities were available from 900 of the study patients. Patients held their hand in the cold water for an average of 46.4 (29.5) seconds. The average NRS at the time of withdrawal was 8.3 (1.8). For further analysis, the time point 30s was chosen because at 30s the mean pain intensity was already high, but the majority (60%) of patients still kept their hand in the water (Table 14).

Table 14. Number of patients who kept their hand immersed in the cold water and their average cold pain intensities.

	15 s	30 s	45 s	60 s	75 s	90 s
Patients (n)	780	545	386	295	238	214
NRS 0 - 10	5.0 (2.5)	6.31 (2.4)	7.1 (2.4)	7.6 (2.3)	7.8 (2.3)	8.0 (2.2)

5.3.1 Associations between cold pain and other phenotypes

The cold pain intensity at 30 s, the total time patients kept their hand immersed or heat pain did not associate with post-operative oxycodone plasma concentrations required for analgesia in the linear regression (Study I) (P=0.20 and P=0.34). Nevertheless, cold pain intensity and the total time patients kept their hand in the cold water showed a significant association with post-operative motion pain intensity (Table 15).

5.3.2 Factors affecting cold pain intensity

Factors affecting experimental cold pain intensity at 30s were the patient's anxiety level, age and *FAAH* rs324420 genotype. Each STAI score increased cold pain intensity by 0.46%, while each age year reduced it by 0.48%. Each *FAAH* rs324420 A-allele reduced cold pain intensity by 8.4% (Table 15).

Table 15. The factors associating with experimental cold pain intensity at 30s. Results were obtained using forward type linear regression. Excluded variables are phenotypes tested but determined insignificant. R square (coefficient of determination) for the model is 0.038. Beta = standardized coefficients for model, B = Increase in experimental cold pain intensity, STAI = State-Trait Anxiety Inventory, BMI = Body Mass Index, NRS = Numerical Rating Scale (0-10).

	Confidence interval 95%			
Variable	**B**	**Lower**	**Upper**	***P***
Anxiety (STAI)	0.46%	0.08%	0.84%	0.018
FAAH rs324420 genotype	8.40%	1.90%	15.30%	0.011
Age (years)	-0.48%	-0.90%	-0.05%	0.028
Excluded variables	**Beta**			***P***
Weight (kg)	-4.80%			0.280
BMI (kg m^{-2})	1.30%			0.779
Preoperative chronic pain (yes/no)	2.30%			0.603
Smoking (yes/no)	-5.60%			0.198
Preoperative breast pain (NRS 0-10)	3.70%			0.435
OPRM1 rs1799971 genotype	4.10%			0.377

5.3.3 Genetic associations with experimental cold pain

OPRM1 rs1799971 genotype did not associate with cold pain intensity at any studied time point or with the total time patients kept their hand immersed in the water (Study II) (Figure 9). Furthermore, when the other 20 *OPRM1* SNPs were also analysed, no significant associations were determined (unpublished data).

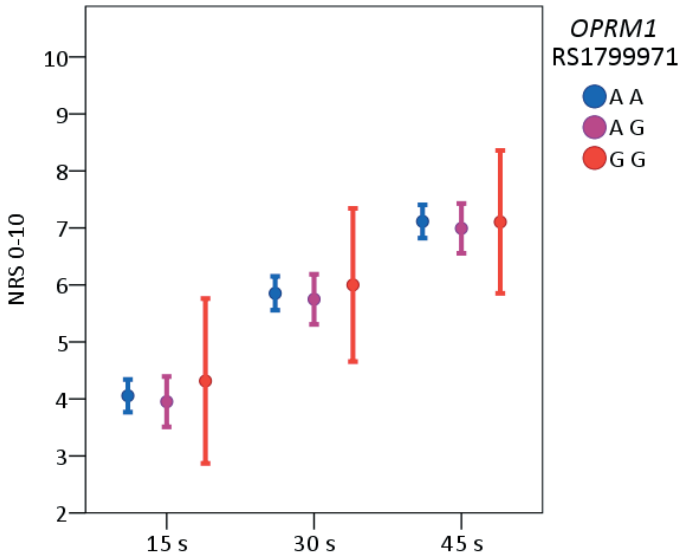

Figure 9. Cold pain intensity by *OPRM1* rs1799971 genotype at different time points

Of the eight studied *FAAH* SNPs, four showed associations with cold pain intensity at 30 s (uncorrected $p < 0.05$). Only two associations survived the Bonferroni correction for multiple testing (corrected $p = 0.0014$). The SNPs rs324420 and rs1571138 were in almost complete linkage disequilibrium (98%). Patients homozygous for the minor allele A of the rs324420 or rs1571138 reported - on average - 1.43 NRS points lower cold pain intensities than patients who were heterozygous or homozygous for the major alleles (Study III)(Figure 10).

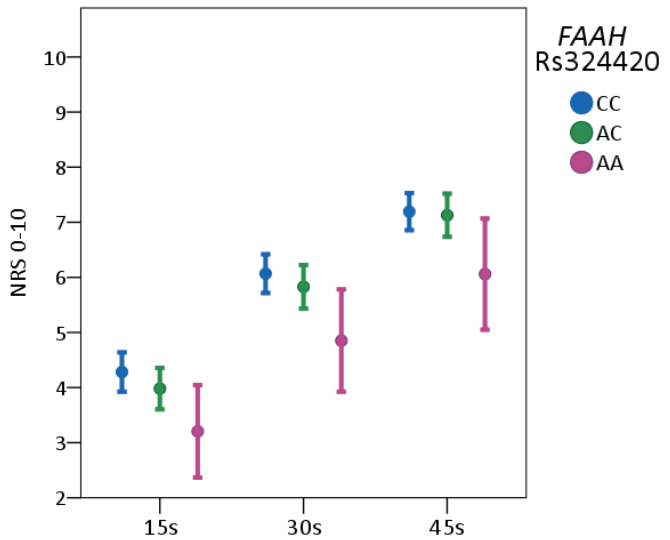

Figure 10. Cold pain intensity by *FAAH* rs324420 genotype at different time points

5.4 Experimental heat pain

Most patients did not consider the sensation caused by the 43°C temperature as painful (mean NRS 0.7). The mean NRS score for heat pain intensity with 48°C temperature for 5 seconds was 3.5 (SD 2.4) of which 95 patients did not regard the sensation as painful; 462 reported mild pain (NRS 1-3); 351 moderate pain (NRS 4-7) and 79 severe pain (NRS 8-10). Heat pain scores were not available for 13 patients (Figure 8).

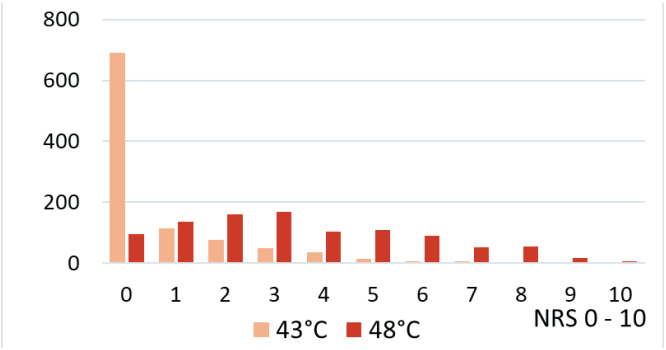

Figure 8. Pain intensities (x-axis) reported by the patient to control (43°C) and heat (48°C) stimulation. Number of patients given on y-axis.

5.4.1 Associations between experimental heat pain and other phenotypes

Experimental heat pain did not associate with post-operative motion pain intensity or analgesic oxycodone dose.

5.4.2 Genetic associations with heat pain

Neither *OPRM1* rs1799971, nor any of the studied *FAAH* SNPs, associated with heat pain intensity at either of the temperatures (Studies II and III). When looking at all the SNPs of *OPRM1*, there were two nominal associations to heat pain intensity: Rs3778153 associated with heat pain when the additive model was used (uncorrected p= 0.043) and rs4870266, when the recessive model was used (uncorrected p= 0.019) for analysis. Neither of the associations remained after corrections for multiple testing.

5.5 Post-operative pain

In the PACU, both rest and motion pain intensities were assessed in all patients before any oxycodone was administered. Pain intensity at rest and in motion correlated almost completely (r = 0.99, P <0.001). Nine patients reported no rest pain (NRS 0); 63 mild rest pain (NRS 1-3); 817 moderate rest pain (NRS 4-7) and 68

severe rest pain (NRS 8-10). Rest pain scores were unavailable for 43 patients. The mean rest pain score was 4.95 (1.47), whereas the mean motion pain score was 4.99 (1.48). Seven patients reported no motion pain, 60 mild motion pain, 815 moderate motion pain and 67 severe motion pain. Motion pain scores were unavailable from 51 patients.

5.5.1 Factors associating with postoperative pain intensity

When looking at factors that explain post-operative motion pain intensity, age was determined to be the most important factor with every age year lowering the pain intensity score by 0.51%. Other significant factors were axillary clearance, preoperative cold pain intensity at 30s and cold pain tolerance. Together these factors explained 8.5% of the variability in the motion pain intensities (Table 15).

OPRM1 rs1799971 genotype did not associate with post-operative motion pain intensity when assessed individually (Study II) or when combined with other variables. *FAAH* did not associate significantly with post-operative motion pain intensity.

Table 15. Factors associating with post-operative motion pain intensity. Results were obtained using forward type linear regression. Excluded variables are phenotypes tested but determined insignificant. R square (coefficient of determination) for the model is 0.085 Beta = standardized coefficients for model, B = Increase in postoperative motion pain intensity, SNB = Sentinel Node Biopsy, STAI = State-Trait Anxiety Inventory, BCS = Breast conserving surgery, BMI = Body Mass Index, NRS = Numerical Rating Scale (0-10).

	Confidence interval 95%			
Variable	**B**	**Lower**	**Upper**	***P***
Age (years)	-0.51%	-0.75%	-0.28%	<0.001
Axillary clearance (vs. SNB)	5.18%	1.83%	8.63%	0.002
Cold pain intensity at 30s (NRS 0-10)	1.83%	0.67%	3.00%	0.002
The total time patients kept their hand immersed in cold water (s)	0.13%	0.02%	0.24%	0.024
Excluded variables	**Beta**			***P***
Weight (kg)	-0.10%			0.983
BMI (kg m^{-2})	-0.30%			0.954
Anxiety (STAI)	5.23%			0.259
Preoperative chronic pain (yes/no)	6.18%			0.182
Smoking (yes/no)	6.18%			0.174
Heat pain intensity (NRS 0-10)	4.81%			0.333
Preoperative breast pain (NRS 0-10)	4.50%			0.34
Mastectomy (vs. BCS)	2.47%			0.592
OPRM1 rs1799971 genotype	-3.53%			0.421
FAAH rs324420 genotype	-5.45%			0.208

5.6 Post-operative oxycodone requirements

Altogether 47 patients did not require any oxycodone in the PACU. Of these, 31 patients did not require oxycodone during the first 20 postoperative hours. On average, the patients required 0.13 mg/kg of oxycodone to feel satisfied with pain relief and a total of 0.25 mg/kg during the first 20 postoperative hours. The time it took the patients to reach satisfactory analgesia varied from 11 to 132 minutes (mean 38.3 min). The time the patients remained satisfied after the first state of satisfactory analgesia varied from 10 to 1106 minutes (mean 161 min). The 399 patients, who did not require more oxycodone after the first state of satisfactory analgesia in the PACU, were older and less anxious compared with the 539 patients who required more oxycodone in the PACU. The oxycodone dose required for first state of satisfactory analgesia did not differ between these two groups. A total of 216 patients did not require more oxycodone in the first 20 postoperative hours.

5.6.1 Genetic association with postoperative oxycodone requirements

OPRM1 rs1799971 associated with the oxycodone dose required for the first state of satisfactory analgesia. The patients homozygous for the minor allele required on average 0.04 mg/kg more oxycodone to achieve analgesia than the major allele homozygotes (Study II). No difference between the genotype groups was seen at the total oxycodone consumption during the 20 postoperative hours. *FAAH* SNPs rs3863641, rs3766246, rs324420 and rs1571138 showed nominal associations (uncorrected $p<0.05$) with the oxycodone dose required for the first state of satisfactory analgesia and with the total oxycodone consumption during the first 20 postoperative hours. However, these associations did not survive the corrections for multiple testing (Study III, supplementary material).

5.6.2 Factors explaining post-operative oxycodone requirements

Factors that associate with post-operative oxycodone dose requirement were motion pain intensity before patient was administered oxycodone, BMI, surgery type (axillary clearance vs. sentinel node biopsy), age, *OPRM1* genotype and the intensity of preoperative breast pain (Figure 11). Every motion pain NRS point increased the oxycodone dose requirement by 23%. Similarly, patients who underwent axillary clearance or had preoperative breast pain required higher oxycodone doses. Each *OPRM1* rs1799971 G allele increased the required post-operative oxycodone dose by 10.7%. In contrast, high age and BMI were associated with lower oxycodone requirements (Table 16.).

Table 16. Factors associating with the oxycodone dose required for the 1st state of satisfactory analgesia. Results were obtained using forward type linear regression. Excluded variables are phenotypes tested but determined insignificant. R square for the model is 0.286 R Square = coefficient of determination, Beta = standardized coefficients for model, B = Increase in oxycodone dose needed for satisfactory analgesia, SNB = Sentinel Node Biopsy, STAI = State-Trait Anxiety Inventory, BCS = breast conserving surgery, BMI = Body Mass Index, NRS = Numerical Rating Scale (0-10). * *CYP2D6* genotypes include ultra-rapid, extensive, intermediate and poor metabolizers

	Confidence interval 95%			
Variable	**B**	**Lower**	**Upper**	***P***
Motion pain (NRS 0-10)	23.10%	18.10%	28.40%	<0.001
BMI (kg m^{-2})	-2.52%	-3.77%	-1.26%	<0.001
Axillary clearance (vs. SNB)	12.80%	4.02%	22.40%	0.004
Age (years)	-0.78%	-1.38%	-0.18%	0.011
OPRM1 rs1799971 genotype	10.70%	1.75%	18.80%	0.02
Preoperative breast pain (NRS 0-10)	5.21%	0.78%	9.83%	0.021
Excluded variables	**Beta**			***P***
Weight (kg)	-5.91%			0.524
Preoperative chronic pain (no/yes)	0.10%			0.977
Anxiety (STAI)	5.65%			0.172
Smoking (no/yes)	0.10%			0.976
Cold pain intensity at 30s (NRS 0-10)	3.45%			0.405
The total time patients kept their hand immersed in cold water (s)	-3.34%			0.402
Heat pain (NRS 0-10)	4.50%			0.271
Mastectomy (vs. BCS)	6.18%			0.165
Resting pain (NRS 0-10)	36.60%			0.247
FAAH rs324420 genotype	2.02%			0.612
CYP2D6 genotype*	-2.18%			0.576
CYP3A4 rs355367 genotype	1.51%			0.713
CYP3A5 rs776746 genotype	3.67%			0.361

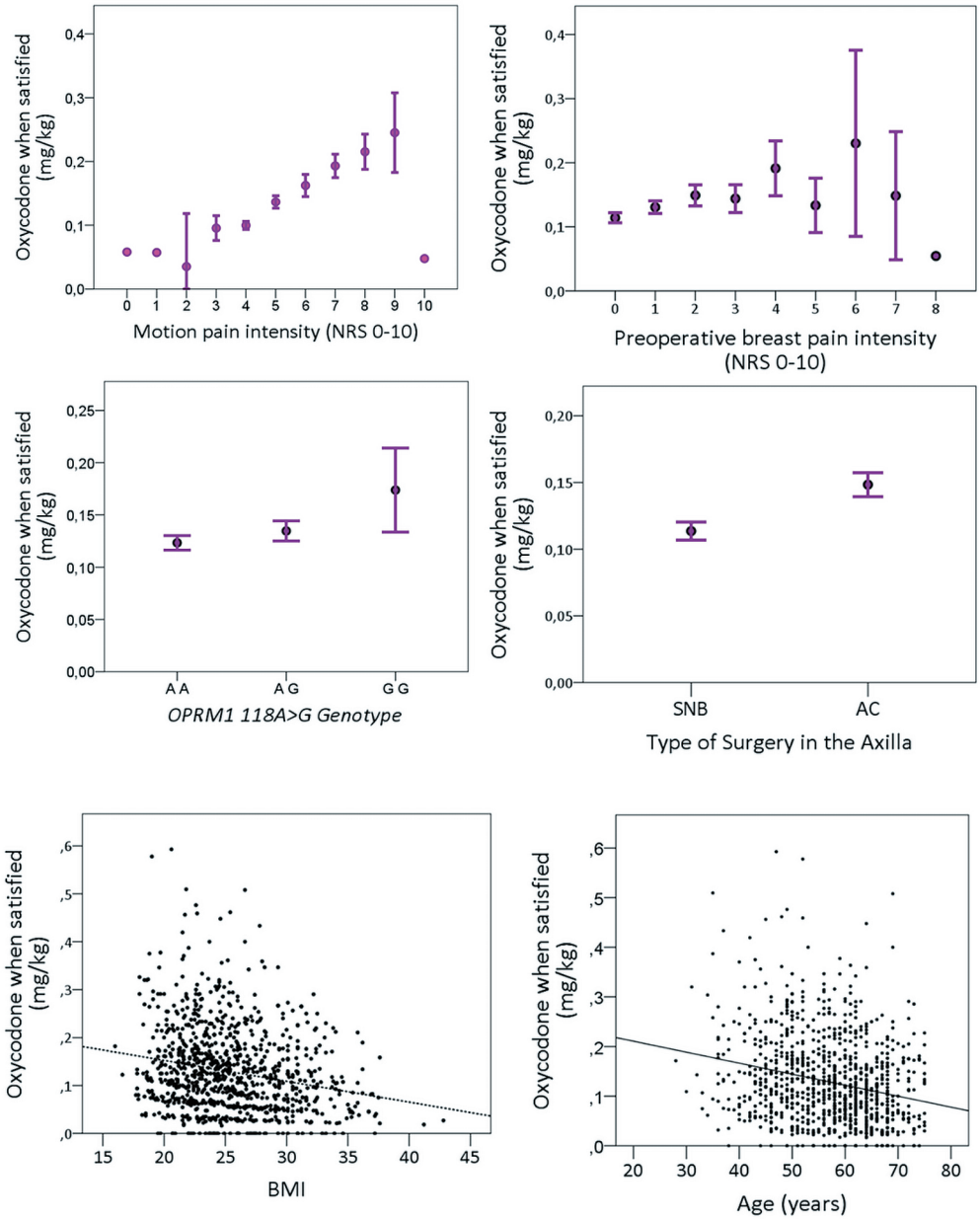

Figure 11. Factors associating with post-operative oxycodone requirement. NRS = numerical rating scale, SNB = sentinel node biopsy, AC = axillary clearance, BMI = body mass index.

5.7 Post-operative oxycodone and metabolite concentrations

Oxycodone and metabolite concentrations were available from 938 patients at the first state of adequate analgesia and from 539 patients at the time they required additional oxycodone. Forty-seven patients did not require oxycodone in the PACU, so their oxycodone concentrations were not determined and further15 patients, oxycodone concentration analyses were unsuccessful.

Oxycodone dose and plasma concentrations correlated significantly (r= 0.599) as did the plasma oxycodone and metabolite concentrations at the two time points (Study I). At the first state of adequate analgesia, the mean oxycodone concentration was 41.3 ng/ml (Table 17). The concentrations of the metabolites of oxycodone were found to be relatively low at the initial measuring point and satisfactory oxycodone plasma concentrations was found to vary greatly between individuals.

Table 17. Oxycodone and metabolite concentrations at the two measurement points.

At the first state of adequate analgesia	Mean (SD) (ng/ml)	Geometric mean (CV%) (ng/ml)	Range (ng/ml)
Oxycodone	41.3 (27.4)	33.3 (66%)	0 – 311
Noroxycodone	2.73 (2.93)	1.46 (106%)	0 - 16.5
Oxymorphone	0.146 (0.207)	0.11 (110%)	0 - 1.2
Noroxymorphone	0.392 (0.720)	0.26 (150%)	0 - 7.6
When patients needed a new dose of oxycodone			
Oxycodone	26.7 (18.3)	21.7 (69%)	0 – 191
Noroxycodone	4.84 (3.57)	3.67 (73%)	0.2 - 19.3
Oxymorphone	0.186 (0.192)	0.14 (89%)	0 - 1.5
Noroxymorphone	0.967 (0.929)	0.67 (92%)	0 - 6.7

Patient's motion pain intensity and type of surgery in the axilla were seen to associate with the oxycodone concentration needed for adequate analgesia (Study I). Each motion pain NRS score increased satisfactory post-operative oxycodone concentration by 21.7%. Axillary clearance increased satisfactory oxycodone concentration by 23.4% when compared to sentinel node biopsy. *OPRM1, CYP3A4/5 or CYP2D6* genotypes did not show any significant association with post-operative oxycodone concentration.

CYP2D6 genotype associated with oxymorphone and noroxymorphone concentrations at the two measuring points (Study I) (Figure 12). As expected, patients with PM genotype showed minimal concentrations, whilst the highest concentrations were seen in patients with UM genotype. In all *CYP2D6* genotype groups, the metabolite concentration remained low compared to oxycodone.

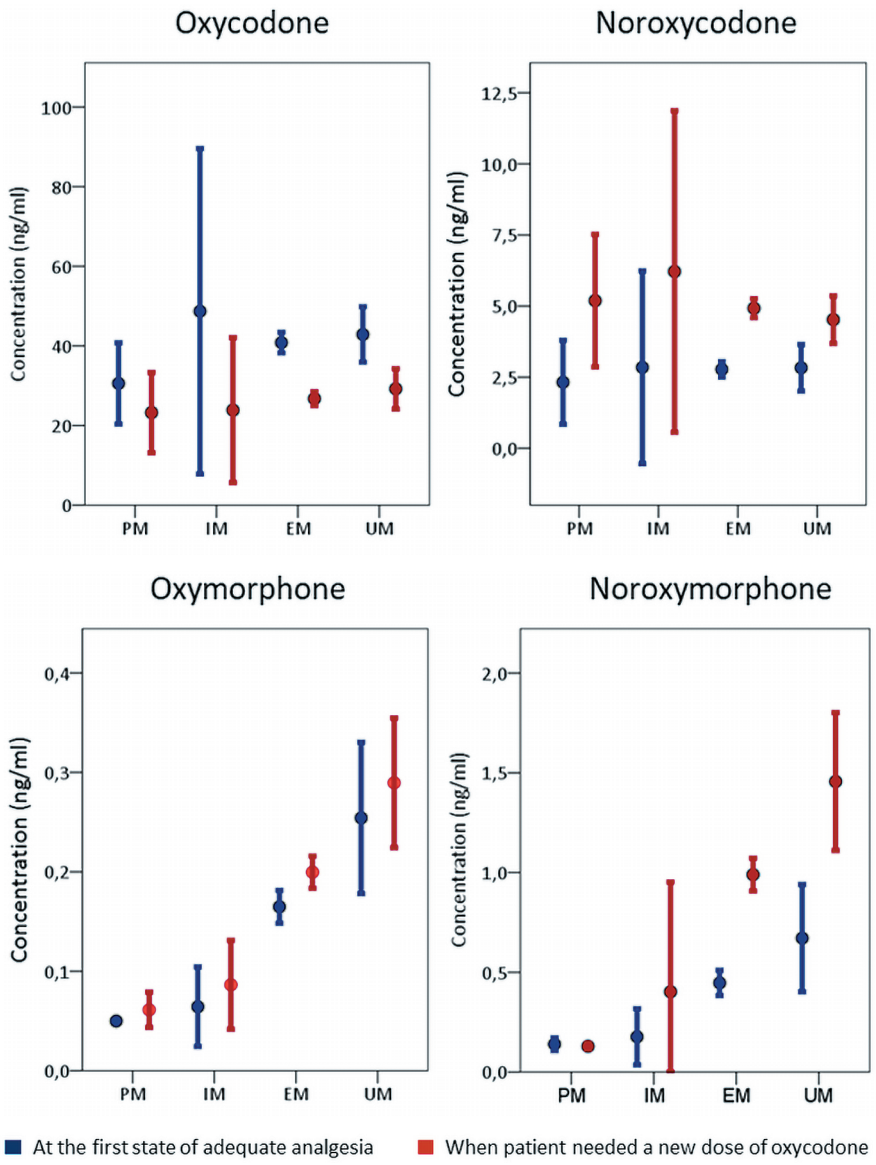

Figure 12. Oxycodone and metabolite concentrations by *CYP2D6* genotype at the two measuring points. PM = poor metabolizer, IM = intermediate metabolizer, EM = extensive metabolizer, UM = ultra-rapid metabolizer.

6. DISCUSSION

6.1 Methodological considerations

6.1.1 Ethical considerations

Ethical questions are particularly relevant when studying pain especially as pain cannot be assessed in study subjects unless they are exposed to some form of pain. The ethical guidelines of The International Association for the Study of Pain state: "*In any pain research, stimuli should never exceed a subject's tolerance limit and subjects should be able to escape or terminate a painful stimulus at will. The minimal intensity of noxious stimulus necessary to achieve goals of the study should be established and not exceeded.*" and "*In all circumstances, including studies that employ placebo and sham treatment methods, an effective, accepted method of pain relief must be provided on request of the patient or subject. The availability of alternative pain relief should be made clear in the consent form and the instruction before the study begins.*"

Our study was approved by two ethics committees of the Helsinki and Uusimaa Hospital District (HU: the Coordinating Ethics Committee and the Ethics Committee of the Department of Surgery). All patients volunteered to participate in the study and gave written informed consent. The pain inflicted to the patients during cold and heat pain testing was not harmful, the stimulus was of short duration and the patient could terminate the stimulation whenever they chose. In addition, the type of surgery was selected in relation to tumour characteristics, existence of malignant cells in the sentinel node and the patients' wishes. All patients received standardised anaesthesia but, if the patient's status so required, the protocol was violated to treat the patient in the best way possible. Moreover, patients whose surgery or anaesthesia had to be changed were excluded from the study. Post-operatively, all patients were titrated to satisfactory analgesia and all patients under study were not subjected to any additional pain or suffering when compared with similar patients undergoing surgery for breast cancer. On the contrary, they were monitored exceptionally well since all study patients were treated by their own anaesthesia nurse.

6.1.2 The study cohort

The study sample of 1,000 women is large when compared with previous studies that have investigated the factors affecting post-operative pain and analgesia and exceptionally substantial compared with those focusing on the effect of specific

genes on these phenotypes. As Table 3 shows, most of the studies that have focused on *OPRM1* and post-operative opioid requirement have been conducted with less than 300 patients. Nevertheless, when considering the multidimensional aspects of pain, an even larger study sample would be required to examine in more detail the individual factors affecting pain sensation and their magnitudes. The multitude of confounding factors regarding pain make it a hard phenomenon to study.

To our knowledge, this study is the first to address the effect of *FAAH* on post-operative pain intensity and oxycodone requirements. Kim et al first demonstrated the association between *FAAH* polymorphisms and cold pain in a relatively large sample of 735 individuals, however, their study population contained higher levels of ethnic variation (Kim et al., 2006).

In contrast, the current study patients were a relatively homogenous group - all patients were female and the vast majority of them were of Finnish origin (992 women). The surgical procedures included in the study consisted of four different types of surgery: In the breast area, either conserving surgery or mastectomy was conducted, whereas in the axilla all patients underwent sentinel node biopsy. If malignant cells were identified in the sentinel node, axillary clearance was performed to remove all lymph nodes to eradicate cancerous cells. As a result, the confounding factors in this study were determined to be minimal. The kidney and liver functions of the patients were not analysed. Both could have provided important information considering the metabolism and clearance of oxycodone.

6.1.3 The study procedure and genotyping

All study patients underwent the test in the same order: The day before surgery, all demographic and psychological data were collected and experimental cold and heat pain tests conducted. Furthermore, the anaesthesia protocol used was standardized for all patients. In the PACU, all patients were tested for both rest and motion pain before any oxycodone was administered. Nevertheless, it should be noted that the time patients required in order to reach satisfactory anaesthesia varied from 11 minutes to 132 minutes which could have affected the oxycodone and metabolite concentrations. Similarly, the duration of satisfactory analgesia varied from 10 minutes to 18h.

All genotyping was undertaken such that it as blind to patient phenotype. All genotypes were in Hardy-Weinberg equilibrium, which indicates that the study sample was large enough and there was no selective pressure on any of the alleles studied. Thus the results are comparable with other populations.

In our study the analgesic oxycodone concentration was based on the patients' own report of satisfactory analgesia, which still is the most accurate measurement of pain intensity. However, many confounding factors known to affect the

experience of pain and analgesic requirement might have affected the results. The research nurse asked about the pain every 5 minutes instead of the patient reporting the exact time she reached satisfactory analgesia. Once the patient indicated satisfactory analgesia, no further oxycodone was administered until she indicated needing a new dose of oxycodone. Analgesic oxycodone concentrations varied extensively as did the concentrations at which the patients needed more oxycodone. The great interindividual variation in the analgesic concentration observed in this study is further evidence for the importance of personalized post-operative analgesia. It must also be noted that the adverse effects of oxycodone, such as nausea and delirium, can occur already at concentrations that are below the analgesic concentrations.

6.1.4 Statistical analysis and statistic and clinical significance

Studies II and III were sufficiently powered - excluding one rare *FAAH* SNP - which ensures that the study sample was of appropriate size to observe the expected effect sizes. Nonetheless, power calculations are always based on many assumptions, which increases the risk for misrepresentations in conclusions. The most common error is an overestimation of effect size that leads to an underestimation of the study population size. This, in turn, could lead to a failure in the identification of an association and thus a false conclusion. All genotype-phenotype associations were conservatively corrected for multiple testing in order to minimise the probability of coincidental association.

Statistical significance was set at corrected p-value <0.05, while results with uncorrected p-value <0.05 were considered nominally significant. Furthermore, it has to be acknowledged that statistically significant results do not - necessarily - imply that the results are significant in the clinical context.

6.2 Factors explaining experimental pain intensities

In the linear regression model conducted in this thesis work anxiety level, age and *FAAH* rs324420 genotype were shown to significantly explain the perceived experimental cold pain intensity. Age reduced the reported pain intensity on average by 0.48% for every year, whereas anxiety and rs324420 G-allele increased the perceived pain intensity by 0.46% per STAI score and 8.4% for each G-allele. Together these factors explained 3.8% of the total variance between study patients.

Age has previously been associated with reduced post-operative pain intensity, but studies focusing on experimental pain intensities are few. Kim et al investigated cold and heat pain intensity with a population of 617 individuals aged between 16 to 66 years and found no association between experimental pain intensities and age (Kim et al., 2004). Considering the quite small effect age had on reported experimental

cold pain intensity in our study, it is likely that the association could be missed in studies with a smaller study population or less extensive age range.

Anxious individuals report higher pain intensities and pay more attention to painful stimuli (Eccleston et al., 1999; Keogh et al., 2002). Studies have shown that anxiety can increase the reported intensity of cold pain (Al Absi et al., 1991; Jones et al., 2002) and in both studies anxiety was provoked prior to the cold pressor test. fMRI imaging showed that pain related anxiety can increase perceived pain intensity and that this activates the entorhinal cortex of the hippocampal formation (Ploghaus et al., 2001). In a study by Schidt et al., panic disorder patients reported higher anxiety sensitivity before a cold pressor test and both higher pain and anxiety intensities during the test than healthy controls (Schmidt et al. 1999). In this thesis, anxiety was associated with higher experimental cold pain intensities even though the majority of the patients had low anxiety according to the STAI scores (Kaunisto et al., 2013).

FAAH rs324420 genotype also associated with cold pain intensity with the minor allele homozygotes reporting the lowest intensities. Four *FAAH* SNPs showed either nominal (uncorrected $p < 0.05$) or significant (corrected $p < 0.05$) associations with cold pain in Study II. The two SNPs that produced significant association were rs324420 and rs1571138 and these two SNPs were shown to be in almost complete linkage disequilibrium. The SNPs producing nominal associations in the GWA analysis were rs3766246 and rs4660928 and these associations did not survive correction for multiple testing.

In a previous study by Kim et al., rs324420 did not show any association with cold pain, although the same study found significant associations between cold pain intensity and three SNPs (rs932816, rs4141964, rs2295633). Based on our own preliminary targeted SNP analysis, rs4141964 also showed significant associations with cold pain intensity, heat pain intensity and oxycodone consumption. Unfortunately, the SNP was not found in the GWA data and so it was not included in the GWA analysis.

All SNPs found to associate with cold pain (rs324420, rs4141964, rs3766426 and rs4660928) are located within 6 kb of each other. Rs324420 and rs3766426 were shown to be in 64% linkage disequilibrium as well as rs324420 and rs4660928 (Study III). Rs4141964 was not included in this analysis due to its unavailability in the GWA data. Nevertheless, rs4141964 and rs324420 have been shown to locate in the same haploblock in a study by Bidwell et al. (2013). Therefore, any of these SNPs or some other SNP in strong linkage disequilibrium with them could cause the association signal. The fact that rs324420 causes an amino acid change makes it the most likely SNP to cause these associations, however, the impact of this variant on the protein configuration has not been fully verified. Chiang et al. (2004) did determine that in rs324420 A/A individuals the FAAH expression in T lymphocytes

was reduced to less than 50% compared with wild-type individuals. The reduction in expression was shown not to be cell-type specific. When transcription/translation efficiencies were compared, the change in expression seemed to be due to deficient post-translational folding of the protein, which led to cellular destabilization of the enzyme (Chiang et al., 2004). Other possible explanatory factors for the different expression levels include alternative splicing.

Reduced FAAH expression could lead to increased anandamide concentrations in the synaptic cleft, which in turn would lead to increased activation of the CB_1 and CB_2 receptors. However, as anandamide is only a weak agonist for the CB_1 receptor compared with 2-AG, the magnitude of change in total CB_1 activations remains uncertain. Moreover, as anandamide also has another metabolic pathway through COX-2, any change in the anandamide concentration in the synaptic cleft is a sum of the changes in these pathways.

In this work, *OPRM1* SNPs did not associate with either heat or cold pain intensities or cold pain withdrawal, even though weak association signals were determined for (uncorrected 0.01 <p> 0.05) of rs3778153 and rs4870266 with heat pain. Previously *OPRM1* rs1799971 has been associated with cold and heat pain thresholds in a paediatric population where G-allele carriers presented with higher thresholds for both heat and cold pain (Matic et al., 2016). In another study that focused on the effect of the gene polymorphism on multiple experimental pain models, no association between *OPRM1* SNPs and cold or heat pain was seen - Rs1799971 was included in the analysis (Nielsen, 2016).

6.3 Factors affecting post-operative pain intensity

In the linear regression model conducted in this thesis, the factors affecting post-operative motion pain intensity were age, type of axillary surgery (SNB vs. AC), cold pain intensity at 30s and total time the patients kept their hand immersed in the cold water. Neither *OPRM1* nor *FAAH* genotypes associated with post-operative motion pain intensity. Post-operative rest and motion pain intensities correlated almost completely (r= 0.99). A previous descriptive study from our cohort reported age, type of surgery and heat pain intensity as significant factors for this same phenotype (Kaunisto et al., 2013). There were some differences between the statistical methodology and tested variables between these two studies, which may explain why cold pain intensity and tolerance replaced heat pain intensity in this analysis. The effect of heat pain in the descriptive study was relatively weak when compared with the associations of age and type of surgery.

There are several previous studies where older age has been associated with lower reported post-operative pain intensities (Ip, 2009) and although numerous factors have been hypothesized to cause this phenomenon, thus far no definitive explanation has been found.

Type of surgery had a significant effect on postoperative pain intensity, as previously shown e.g. by Gerbershagen et al. (2013). In our study, the type of axillary surgery proved to be a significant factor associated with acute postoperative pain intensity, while the type of surgery in the breast area did not show any significant association. Axillary clearance is performed when cancerous cells are found in the sentinel lymph node and the procedure aims to decrease the risk for cancer reoccurrence in the axilla and metastases. Nevertheless, the removal of lymph nodes in the axilla requires major surgery in an area highly innervated with nerves (Henry et al., 2017). Furthermore, the axilla also presents a high number of anatomical variations that make surgery more difficult and subject patients to complications such as vascular and nerve injury (Khan et al., 2012). Axillary clearance has been associated with higher occurrence of post-operative chronic pain and the development of lymphedema in the arm distal from the operation site (Göker et al., 2013; Bruce et al., 2014; Miguel et al., 2001). To the best of our knowledge, there are no previous studies that address the effect of axillary clearance on acute post-operative pain intensity.

Both noxious cold stimulation and nociceptive pain induced by tissue damage during surgery activate the spinothalamic and spinoreticular tracts in the spinal cord. The spinothalamic route is hypothesized to function as the major pathway for the discriminatory-sensory component of pain, while there is evidence of affective-motivational and pain modulating activities as well. The spinoreticular tract is also hypothesized to participate in the motivational-affective component of pain (Millan 1999). In the brain, cold pain has been shown to activate medial and lateral thalamus, parieto-insular cortex, anterior cingulate cortex and primary and secondary somatosentory cortex (Maihöfner et al., 2002; Boivie et al., 1989; Greenspan et al., 1999; Schmahmann et al., 1992; Davis et al., 1999; Craig et al., 2000; Craig et al., 1996; Davis et al., 1998; Casey et al., 1996). Injury-related nociceptive pain, such as post-operative pain, has been demonstrated to activate PAG, hypothalamus, prefrontal cortex, insula, anterior cingulate cortex, posterior parietal cortex, primary somatosensory and motor cortexes, supplementary motor area and cerebellum (Hsieh et al., 1996).

It has been hypothesized that experimental cold pain intensity could correlate and therefore predict post-operative pain intensity. So far, the studies that have investigated this correlation have produced mixed results: In two small studies (20 and 54 patients), cold pain showed no correlation with post-operative pain (Lautenbacher et al., 2009; Martinez et al., 2007). In contrast, Bisgaard et al. did find a significant association between cold pain tolerance and post-operative pain intensity when they studied 150 patients undergoing cholecystectomy (Bisgaard et al., 2001). This could indicate that to identify association between cold pain and post-operative pain requires a large study sample for the test results to reach statistical significance. In our study, each cold pain intensity NRS score raised the post-operative NRS score by 1.8% and each second of cold pain tolerance by 0.13%.

6.4 Factors affecting the oxycodone dose required for satisfactory analgesia

In the analysis performed as part of this thesis, the factors associated with post-operative oxycodone dose required for satisfactory analgesia were post-operative motion pain intensity, BMI, type of surgery in the axilla, *OPRM1* rs1799971 genotype, age and preoperative breast pain during the week prior to surgery. Together these factors explained almost 29% of the interpatient variation in oxycodone requirement, however, a proper therapeutic range for oxycodone could not be determined since both the oxycodone doses and concentrations varied substantially.

The major predictor for post-operative oxycodone requirement was post-operative motion pain intensity with every NRS point increasing the required oxycodone dose by 23.1%. This indicated that post-operative pain intensity could be used as a clinical sign that predicts part of the oxycodone dose required for satisfactory analgesia. In addition, those patients who reported the highest pain intensities also required the largest doses of post-operative oxycodone. This knowledge can be used to titrate patients to adequate analgesia faster.

The patient related factors associating with the required oxycodone dose were age, BMI and *OPRM1* rs1799971 genotype. Older age and BMI associated with lower doses of oxycodone, whilst *OPRM1* rs1799971 GG-homozygotes required on average 0.04 mg/kg more than A-allele carriers did.

The negative correlation between age and analgesic consumption has been noticed in many studies and in a review that investigated the factors affecting post-operative pain intensity and analgesic consumption (Ip et al., 2009). Of the eight studies included, six found associations with age and analgesic consumption, while two studies presented conflicting results. The reduced opioid requirement has been hypothesized to be due to reduced metabolism and excretion of opioids (Liukas et al., 2011). Furthermore, as elderly people also have on average less lean body mass this could affect the distribution of oxycodone in the body. Oxycodone resembles morphine in being less lipophilic than fentanyl and buprenorphine (Pöyhiä et al., 1994).

OPRM1 rs1799971 genotype was shown to be associated with post-operative oxycodone dose requirement in both Study II and in the stepwise linear regression (Table 16.) The putative effect of *OPRM1* rs1799971 could be due to the altered half-life and endorphin binding potential of the mutated receptor. The mutated receptor is less stable when compared with the wild-type receptor (Huang et al., 2012). As discovered by Bond et al., it binds β-endorphin 3 times more effectively than the wild type receptor (Bond et al., 1998). Therefore, the overall effect of *OPRM1* rs1799971 is offset by the reduced expression of the receptor and the higher binding affinity of some opioids. It must be noted that even though β-endorphin showed a higher affinity to the mu-opioid receptor, other opioids including morphine, encephalin and

dynorphin showed no changes in receptor binding. Oxycodone was not tested (Bond et al., 1998).

The *OPRM1* rs1799971 G-allele has been shown to increase post-operative opioid requirements in three previous large studies (Tan et al., 2009, Sia et al., 2013, Sia et al., 2008), whereas in smaller studies, the results have been inconclusive. Only two prior studies (266 and 48 patients) have assessed the effect of *OPRM1* rs1799971 on oxycodone requirements and both found no association (Zwisler at al., 2012, Bruehl at al., 2006). A possible explanation for the conflicting results might be the alternative splicing and epigenetics which confound the effect on OPRM1 rs1799971 (Oertel et al., 2012). In our Study II, the patients who were homozygous for the G-allele required - on average - 0.16 mg/kg of oxycodone, while AA individuals required 0.12mg/kg and AG individuals 0.13mg/kg.

Axillary clearance was associated with higher oxycodone requirements as well as higher post-operative motion pain intensities. However, as motion pain intensity was also an independent factor associated with post-operative oxycodone requirement, axillary clearance has an indirect effect on oxycodone dose requirement.

Previously, preoperative pain has been associated both with post-operative pain intensity and post-operative analgesic consumption (Ip et al., 2009). In our study, preoperative breast pain during the week before surgery associated with post-operative oxycodone requirement but not with post-operative motion pain intensity.

Surprisingly, anxiety did not associate with either post-operative pain intensity or analgesia requirement, even though previously anxiety has been shown to associate with higher post-operative intensities and analgesic requirements. Opioids, including oxycodone, have anxiolytic effects, which could lead to anxious patients demanding higher doses to suppress anxiety. Generally, our patients reported quite low anxiety scores, which could explain why anxiety did not show any significant associations to post-operative outcomes (Kaunisto et al. 2013).

6.5 Oxycodone and metabolite concentrations

The factors that associated with post-operative oxycodone plasma concentration required for satisfactory analgesia were post-operative motion pain intensity and type of surgery in the axilla (Study I).

The analgesic oxycodone concentrations varied remarkably. The oxycodone dose and concentration correlated highly but not completely (R^2= 0.599, Study I). Even with the same oxycodone dose, the analgesic concentration varied up to fourfold and as such, no general analgesic concentration could be determined.

The other factors (BMI, Age, *OPRM1* genotype and preoperative breast pain) associated with the oxycodone dose required for satisfactory analgesia did not show

any association with nalgesic oxycodone concentrations. The lack of association of age and BMI could indicate that the oxycodone given stayed in the plasma and did not absorb to other tissues (excluding the CSF). In general, elderly people have a reduced total blood volume (Messerli et al., 1983), which could explain the higher concentrations received with smaller doses. High BMI caused by excess body fat does not affect plasma concentrations unless the measured molecule is lipophilic, thus the effect on oxycodone is minimal as it is a hydrophilic molecule (Poyhia et al., 1994).

As expected, CYP2D6 genotype affected the plasma concentrations of oxymorphone and noroxymorphone at both studied measurement points. Patients with the UM genotype showed over three times higher oxymorphone concentrations when compared to the patients with PM genotype at both measurement points. Oxymorphone has been shown to have a high affinity to the mu-opioid receptor (Lalovic et al., 2006), therefore, the increased metabolite formation associated with CYP2D6 UM-genotype could reduce the analgesic oxycodone requirement. Nevertheless, the CYP2D6 genotype did not affect the oxycodone dose or plasma concentration required for satisfactory analgesia, which could be due the low concentration of oxymorphone even in CYP2D6 ultra-rapid metabolizers (Study I) and partly to the differences in BBB penetration. To our knowledge, no human data exists but with mice the CSF concentration of oxycodone has been shown to be twice as high compared to plasma concentration 60 minutes after dosing. For oxymorphone the ratio was only 0.23 (Lalovic et al., 2006), which indicates that oxycodone reaches the mu-opioid receptors more easily and overcomes the differences caused by diverse binding potentials.

6.6 Future perspectives

This thesis work offers a view into factors affecting experimental and post-operative pain sensitivity and post-operative analgesics requirements. Better knowledge of these factors would offer important tools for clinicians to predict the patients' post-operative pain intensity and opioid requirement. Especially data of the estimated effects of the statistically significant explanatory factors is valuable information for clinicians who treat post-operative pain. Even though this study was able to identify several significant explanatoryfactors for each of the studied pain variables, the coefficient of determination (*i.e.* the proportion of the variance in the dependent variable that is predictable from the independent variable) for each of the regression models was quite low. As a result, there has to be other, still yet unidentified clinical, demographic and/or genetic factors that affect the investigated phenotypes. It is likely that even larger sample sizes of high quality are required to identify these factors.

Development of personalized medicine is one of the major goals for the 21st century and in order to achieve such individualized treatment a massive amount of genomic

data must be collected and analysed. This study proves that *OPRM1* rs1799971 does have an effect on the amount of oxycodone patients require post-operatively, but that this difference is small from the clinical perspective. On the other hand, the results demonstrate that major differences in opioid requirements exist and that some part of this variance is very likely due to genetic factors. The next major step would be to perform a GWAS with sufficient study sample, unified surgical procedures and a replication material, however production of high quality GWAS requires huge resources and collaboration of multiple research centres.

This study also provided evidence of FAAH's effect on cold pain intensity. Unfortunately, no significant association with any of the studied post-operative phenotypes were noticed. The results indicate that at least common variation within the *FAAH* gene is not a major player in modulating post-operative pain and analgesia. This finding could prove to be valuable information when considering the growing interest in the cannabinoid system.

7. CONCLUSIONS

Conclusions addressing the aims of this thesis:

I. Post-operative pain intensities and analgesic concentrations of oxycodone showed significant interindividual variation.

II. The most important factor affecting post-operative oxycodone requirement after breast cancer surgery was post-operative pain intensity. The magnitude of this effect was significant both statistically and clinically. Other factors associated with post-operative oxycodone dose requirements were type of surgery, BMI, age, *OPRM1* rs1799971 genotype and the intensity of preoperative pain.

III. The major factors affecting post-operative pain intensity after breast cancer surgery were age and type of surgery. The magnitude of these effects was significant both statistically and clinically. Results of the experimental pain tests performed before the surgery – specifically cold pain intensity and tolerance – showed a weak correlation with post-operative pain intensity, but due to the low level of this effect, the correlation was regarded as having minor clinical significance.

IV. Anxiety, *FAAH* rs324420 genotype and age associated with experimental cold pain intensity.

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REFERENCES

IASP Taxonomy, 2018, Pain Terms: Pain (https://www.iasp-pain.org/Education/Content.aspx?ItemNumber=1698#Pain)

NCBI Gene Database: *OPRM1*

Opioid Receptor Mu 1 [Homo Sapiens (Human)] https://www.ncbi.nlm.nih.gov/gene/4988 (accessed 10/2017)

1000 Genomes Browser (phase3) https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/?assm=GCF_000001405.25 (accessed 10/2017)

CYP3A4 Allele Nomenclature

http://www.cypalleles.ki.se/cyp3a4.htm (accessed 11/2017).

NCBI Variation Viewer https://www.ncbi.nlm.nih.gov/variation/view/?q=2166%5Bgeneid%5D (accessed 10/2017).

NCB1 Gene Database: CYP3A4

Cytochrome P450 Family 3 Subfamily A Member 4 [Homo Sapiens (Human)] https://www.ncbi.nlm.nih.gov/gene/1576 (accessed 11/2017).

NCBI Gene Database: CYP3A5

Cytochrome P450 Family 3 Subfamily A Member 5 [Homo Sapiens (Human)] https://www.ncbi.nlm.nih.gov/gene/1577 (accessed 11/2017).

NCBI Gene Database: CYP2D6

Cytochrome P450 Family 2 Subfamily D Member 6 [Homo Sapiens (Human)] https://www.ncbi.nlm.nih.gov/gene/1565 (accessed 11/2017)

Sequencing Initiative Suomi Project (SISu), Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Finland, SISu v4.1. http://sisuproject.fi (accessed 11/2017)

Aberle, J., Fedderwitz, I., Klages, N., George, E., Beil, F.U. 2007 Genetic Variation in Two Proteins of the Endocannabinoid System and their Influence on Body Mass Index and Metabolism Under Low Fat Diet. *Hormone & Metabolic Research,* **39** (5) pp. 395-397.

Ahn, K. Smith, S.E., Liimatta, M.B., Beidler, D., Sadagopan, N., Dudley, D.T., Young, T., Wren, P., Zhang, Y., Swaney, S., Van Becelaere, K., Blankman J.L., Nomuda D.K., Bhattachar, S.N., Stiff, C., Nomanbhoy, T.K., Weerapana, E., Johnson, D.S., Cravatt, B.F., 2011 Mechanistic and Pharmacological Characterization of PF-04457845: A Highly Potent and Selective Fatty Acid Amide Hydrolase Inhibitor that Reduces Inflammatory and Noninflammatory Pain, *The Journal of Pharmacology and Experimental Therapeutics,* **338** (1), pp. 114-124.

Al Absi M., and Rokke P.D., 1991 Can Anxiety Help Us Tolerate Pain? *Pain* **46** (1), pp. 43-51.

Alhouayek, M., Muccioli G.G., 2012 The Endocannabinoid System in Inflammatory Bowel Diseases: From Pathophysiology to Therapeutic Opportunity, *Trends in Molecular Medicine,* **18** (10), pp. 615-625.

Almeida T.F., Roizenblatt S., and Tufik S., 2004 Afferent Pain Pathways: A Neuroanatomical Review. *Brain Research* **1000** (1), pp. 40-56.

Arias, A.J., Chan, G., Gelernter, J., Farrer, L., Kranzler, H.R., 2012 Variation in *OPRM1* and Risk of Suicidal Behavior in Drug-Dependent Individuals. *American Journal on Addictions,* **21** (1) pp. 5-10.

Backlund M., Lindgren L., Kajimoto Y., and Rosenberg P.H., 1997 Comparison of Epidural Morphine and Oxycodone for Pain after Abdominal Surgery. *Journal of Clinical Anesthesia* **9** (1), pp. 30-35.

Baliki, M.N., Geha, P.Y., Apkarian, A.V., Chialvo, D.R., 2008 Beyond Feeling: Chronic Pain Hurts the Brain, Disrupting the Default-Mode Network Dynamics, *The Journal of Neuroscience,* **28** (6), pp. 1398.

Barratt, D. T., Bandak, B., Klepstad, P., Dal, O., Kaasa, S., Christrup L.L., Tuke, J., Somoqyi, A.A., 2014 Genetic, Pathological and Physiological Determinants of Transdermal Fentanyl Pharmacokinetics in 620 Cancer Patients of the EPOS Study, *Pharmacogenetics and Genomics,* **24** (4), pp. 185-194.

Barratt, C., Lai, T., Nashef, L., Valentin, A., Fisniku, L., Moran, N., Asherson, P., Makoff, A., 2006 No Association of Single Nucleotide Polymorphisms in the Micro-Opioid Receptor Subunit Gene with Idiopathic Generalized Epilepsy. *Epilepsia,* **47** (10) pp. 1728-1731.

Basbaum, A.I., Bautista, D.M., Scherrer, G., Julius, D., 2009 Cellular and Molecular Mechanisms of Pain, *Cell,* **139** (2) pp. 267-284.

Bautista, D.M., Siemens, J., Glazer, J.M., Tsuruda, P.R., Basbaus, A.I., Stucky, C.L., Jordt, SE., David, J., 2007 The Menthol Receptor TRPM8 is the Principal Detector of Environmental Cold., *Nature,* **448** (7150), pp. 204-208.

Beck A.T., Ward C.H., Mendelson M., Mock J., Erbaugh J., 1961 An Inventory for Measuring Depression, *Archives of General Psychiatry,* **4**, pp. 561-571.

Benarroch, E.E., 2012 Endogenous Opioid Systems, *Neurology,* **79** (8), pp. 807.

Berrettini, W., 2016 Alcohol Addiction and the Mu-Opioid Receptor, *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* **65** pp. 228-233.

Bidwell, L.C., Metrik, J., McGeary, J., Palmer, R.H.C., Francazio, S., Knopik, V.S., 2013 Impulsivity, Variation in the Cannabinoid Receptor (CNR1) and Fatty Acid Amide Hydrolase (FAAH) Genes, and Marijuana-Related Problems., *Journal of Studies on Alcohol & Drugs,* **74** (6), pp. 867-878.

Bisgaard, T., Klarskov, B., Rosenberg, J., Kehlet, H., 2001 Characteristics and Prediction of Early Pain After Laparoscopic Cholecystectomy., *Pain,* **90** (3), pp. 261-269.

Boivie, J., Leijon, G., Johansson, I., 1989 Central Post-Stroke Pain--a Study of the Mechanisms through Analyses of the Sensory Abnormalities., *Pain,* **37** (2), pp. 173-185.

Bond, C., LaForge, K.S., Tian, M., Melia, D., Zhang, S., Borg, L., Gong, J., Schluger, J., Strong, J.A., Leal, S.M., Tischfield, J.A., Kreek, M.J., Yu, L., 1998 Single-Nucleotide Polymorphism in the Human Mu Opioid Receptor Gene Alters Beta-Endorphin Binding and Activity: Possible Implications for Opiate Addiction., *Proceedings of the National Academy of Sciences of the United States of America,* **95** (16), pp. 9608-9613.

Bortoluzzi, M.C., Manfro, A.R., Nodari, R.J. Jr, Presta, A.A., 2012 Predictive Variables for Postoperative Pain After 520 Consecutive Dental Extraction Surgeries., *General Dentistry,* **60** (1), pp. 58-63.

Bortsov, A.V., Millikan, R.C., Belfer, I., Boortz-Marx, R.L., Arora, H., McLean, S.A., 2012 Mu-Opioid Receptor Gene A118G Polymorphism Predicts Survival in Patients with Breast Cancer. *Anesthesiology,* **116** (4) pp. 896-902.

Boström, E., Hammarlund-Udenaes, M., Simonsson, U.S., 2008 Blood-Brain Barrier Transport Helps to Explain Discrepancies in in Vivo Potency between Oxycodone and Morphine, *Anesthesiology,* **108** (3), pp. 495-505.

Bruce, J., Thornton, A.J., Powell, R., Johnston, M., Wells, M., Heys, S.D., Thompson A.M., Cairns Smith, W., Chambers, W.A., Scott, N.W., 2014 Psychological, Surgical, and Sociodemographic Predictors of Pain Outcomes After Breast Cancer Surgery: A Population-Based Cohort Study, *Pain,* **155** (2), pp. 232-243.

Bruehl, S., Chung, O.Y., Donahue, B.S., Burns, J.W., 2006 Anger Regulation Style, Postoperative Pain, and Relationship to the A118G Mu Opioid Receptor Gene Polymorphism: A Preliminary Study., *Journal of Behavioral Medicine,* **29** (2), pp. 161-169.

Buhler, K., Huertas, E., Echeverry-Alzate, V., Gine, E., Molto, E., Montoliu, L., Lopez-Moreno, J.A., 2014 Risky Alcohol Consumption in Young People is Associated with the Fatty Acid Amide Hydrolase Gene Polymorphism C385A and Affective Rating of Drug Pictures. *Molecular Genetics & Genomics: MGG,* **289** (3) pp. 279-289.

Burk, O., Wojnowski, L., 2004 Cytochrome P450 3A and their Regulation, *Naunyn-Schmiedebergs Archives of Pharmacology,* **369** (1), pp. 105-124.

Bush, W.S., Moore, J.H., 2012 Chapter 11: Genome-Wide Association Studies, *PLoS Computational Biology,* **8** (12), pp. e1002822.

Camilleri, M., Carlson, P., McKinzie, S., Grudell, A., Busciglio, I., Burton, D., Baxter, K., Ryks, M., Zinsmeister, A.R. 2008 Genetic Variation in Endocannabinoid Metabolism, Gastrointestinal Motility, and Sensation. *American Journal of Physiology - Gastrointestinal & Liver Physiology,* **294** (1) pp. G13-9.

Campa, D., Gioia, A., Tomei, A., Poli, P., Barale, R., 2008 Association of ABCB1/MDR1 and *OPRM1* Gene Polymorphisms with Morphine Pain Relief, *Clinical Pharmacology & Therapeutics,* **83** (4), pp. 559-566.

Casey, K.L.., Minoshima, S., Morrow, T.J., Koeppe, R.A., 1996 Comparison of Human Cerebral Activation Pattern during Cutaneous Warmth, Heat Pain, and Deep Cold Pain, *Journal of Neurophysiology,* **76** (1), pp. 571-581.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D. 1997 The Capsaicin Receptor: A Heat-Activated Ion Channel in the Pain Pathway. *Nature,* **389** (6653) pp. 816-824.

Caumo, W., Schmidt, A.P., Schneider, C.N., Bergmann, J., Iwamoto, C.W., Adamatti, L.C., Bandeira, D., Ferreira M.B.C., 2002 Preoperative Predictors of Moderate to Intense Acute Postoperative Pain in Patients Undergoing Abdominal Surgery, *Acta Anaesthesiologica Scandinavica,* **46** (10), pp. 1265-1271.

Cavalli-Sforza, L.L., Bodmer. W.F., 1971 *The Genetics of Human Populations*, New York, Freeman and Co.

Chamorro, A., Marcos, M., Miron-Canelo, J., Pastor, I., Gonzalez-Sarmiento, R., Laso, F., 2012 Association of Micro-Opioid Receptor (*OPRM1*) Gene Polymorphism with Response to Naltrexone in Alcohol Dependence: A Systematic Review and Meta-Analysis, *Addiction Biology,* **17** (3) pp. 505-512.

Chang, S.H., Maney, K.M., Phillips, J.P., Langford, R.M., Mehta, V., 2010 A Comparison of the Respiratory Effects of Oxycodone Versus Morphine: A Randomised, Double-Blind, Placebo-Controlled Investigation*, *Anaesthesia,* **65** (10) pp. 1007-1012.

Chiang, H.L., Chia, Y.Y., Lin H.S., Chen C.H., 2016 The Implications of Tobacco Smoking on Acute Postoperative Pain: A Prospective Observational Study, *Pain Research & Management,* **2016**, article ID 9432493, 7 pages, doi:10.1155/2016/9432493

Chiang, K.P., Gerber,A.L., Sipe, J.C., Cravatt, B.F., 2004 Reduced Cellular Expression and Activity of the P129T Mutant of Human Fatty Acid Amide Hydrolase: Evidence for a Link between Defects in the Endocannabinoid System and Problem Drug use., *Human Molecular Genetics,* **13** (18), pp. 2113-2119.

Chidambaran, V., Pilipenko, V., Spruance, K., Venkatasubramanian, R., Niu, J., Fukuda, T., Mizuno, T., Zhang, K., Kaufman, K., Vinks, A.A., Martin, L.J., Sadhasivam, S. 2017 Fatty Acid Amide Hydrolase-Morphine Interaction Influences Ventilatory Response to Hypercapnia and Postoperative Opioid Outcomes in Children. *Pharmacogenomics,* **18** (2) pp. 143-156.

Chmelikova, M., Pacal, L., Spinarova, L., Vasku, A. 2015 Association of Polymorphisms in the Endocannabinoid System Genes with Myocardial Infarction and Plasma Cholesterol Levels. *Biomedical Papers of the Medical Faculty of Palacky University in Olomouc, Czech Republic,* **159** (4) pp. 535-539.

Chou, W.Y.,Wang, C.H.,Liu, P.H.,Liu, C.C.,Tseng, C.C.,Jawan, B., 2006a Human Opioid Receptor A118G Polymorphism Affects Intravenous Patient-Controlled Analgesia Morphine Consumption After Total Abdominal Hysterectomy, *Anesthesiology,* **105** (2) pp. 334-337.

Chou, W., Yang, L.,Lu, H., Ko, J., Wang, C., Lin, S., Lee, T., Concejero, A., Hsu, C., .2006b Association of μ-Opioid Receptor Gene Polymorphism (A118G) with Variations in Morphine Consumption for Analgesia After Total Knee Arthroplasty, *Acta Anaesthesiologica Scandinavica,* **50** (7) pp. 787-792.

Cieslinska, A., Sienkiewicz-Szlapka, E., Kostyra, E., Fiedorowicz, E., Snarska, J., Wronski, K., Tenderenda, M., Jarmolowska, B., Matysiewicz, M., 2015 Mu-Opioid Receptor Gene (*OPRM1*) Polymorphism in Patients with Breast Cancer. *Tumour Biology,* **36** (6) pp. 4655-4660.

Coghill, R. C., McHaffie, J.G., Yen, Y.F., 2003 Neural Correlates of Interindividual Differences in the Subjective Experience of Pain, *Proceedings of the National Academy of Sciences of the United States of America,* **100** (14), pp. 8538-8542.

Colasanti, A., Rabiner, E.A., Lingford-Hughes, A., Nutt, D.J., 2011 Opioids and Anxiety, *Journal of Psychopharmacology (Oxford, England),* **25** (11) pp. 1415-1433.

Conzelmann, A., Reif, A., Jacob, C., Weyers, P., Lesch, K., Lutz, B., Pauli, P. 2012 A Polymorphism in the Gene of the Endocannabinoid-Degrading Enzyme FAAH (FAAH C385A) is Associated with Emotional-Motivational Reactivity. *Psychopharmacology,* **224** (4) pp. 573-579.

Cook-Sather, S.D., Li, J., Goebel T.K., Sussman, E.M., Rehman M.A. Hakonarson, H., 2014 TAOK3, a Novel Genome-Wide Association Study Locus Associated with Morphine Requirement and Postoperative Pain in a Retrospective Pediatric Day Surgery Population, *Pain,* **155** (9), pp. 1773-1783.

Cooper, A.J., Rickels, K., Lohoff, F.W.2013 Association Analysis between the A118G Polymorphism in the *OPRM1* Gene and Treatment Response to Venlafaxine XR in Generalized Anxiety Disorder. *Human Psychopharmacology,* **28** (3) pp. 258-262.

Coulbault, L., Beaussier, M., Verstuyft, C., Weickmans, H., Dubert, L., Tregouet, D., Descot, C., Parc, Y., Lienhart, A., Jaillon, P., Becquemont, L. 2006 Environmental and Genetic Factors Associated with Morphine Response in the Postoperative Period. *Clinical Pharmacology & Therapeutics,* **79** (4) pp. 316-324.

Craig, A.D., Chen, K., Bandy, D., Reiman E.M., 2000 Thermosensory Activation of Insular Cortex, *Nature Neuroscience,* **3** (2), pp. 184-190.

Craig, A.D., Reiman, E.M., Evans, A., Brushnell, M.C. 1996 Functional Imaging of an Illusion of Pain, *Nature,* **384** (6606), pp. 258-260.

Cravatt, B.F., Demarest, K., Patricelli, M.P., Bracey, M.H., Giang, D.K., Martin, B.R., Lichtman, A.H., 2001 Supersensitivity to Anandamide and Enhanced Endogenous Cannabinoid Signaling in Mice Lacking Fatty Acid Amide Hydrolase, *Proceedings of the National Academy of Sciences of the United States of America,* **98** (16), pp. 9371-9376.

Dai, D., Tang, J., Rose, R., Hodgson, E., Bienstock, R.J., Mohrenweiser, H.W., Goldstein, J.A. 2001 Identification of Variants of CYP3A4 and Characterization of their Abilities to Metabolize Testosterone and Chlorpyrifos, *The Journal of Pharmacology and Experimental Therapeutics,* **299** (3) pp. 825-831.

Daut R.L., Cleeland C.S., Flanery R.C. 1983 Development of the Wisconsin Brief Pain Questionnaire to Assess Pain in Cancer and Other Diseases. *Pain* **17** pp. 197-210.

Davis, C., Loxton, N.J., 2014 A Psycho-Genetic Study of Hedonic Responsiveness in Relation to "Food Addiction". *Nutrients,* **6** (10) pp. 4338-4353.

Davis, C., Zai, C., Levitan, R.D., Kaplan, A.S., Carter, J.C., Reid-Westoby, C., Curtis, C., Wight, K., Kennedy, J.L., 2011 Opiates, Overeating and Obesity: A Psychogenetic Analysis. *International Journal of Obesity,* **35** (10) pp. 1347-1354.

Davis, C.A., Levitan, R.D., Reid, C., Carter, J.C., Kaplan, A.S., Patte, K.A., King, N., Curtis, C., Kennedy, J.L., 2009 Dopamine for "Wanting" and

Opioids for "Liking": A Comparison of Obese Adults with and without Binge Eating. *Obesity,* **17** (6) pp. 1220-1225.

Davis, K.D., Lozano, R.M., Manduch, M., Tasker, R.R., Kiss, Z.H., Dostrovsky, J.O., 1999 Thalamic Relay Site for Cold Perception in Humans., *Journal of Neurophysiology,* **81** (4), pp. 1970-1973.

Davis, K.D., Kwan, C.L., Crawley, A.P., Mikulis, D.J., 1998 Functional MRI Study of Thalamic and Cortical Activations Evoked by Cutaneous Heat, Cold, and Tactile Stimuli., *Journal of Neurophysiology,* **80** (3), pp. 1533-1546.

Dayer, P., Desmeules, J., Leemann, T., Striberni, R., 1988 Bioactivation of the Narcotic Drug Codeine in Human Liver is Mediated by the Polymorphic Monooxygenase Catalyzing Debrisoquine 4-Hydroxylation (Cytochrome P-450 dbl/bufI), *Biochemical and Biophysical Research Communications,* **152** (1) pp. 411-416

De Gregori M., Diatchenko L., Ingelmo P.M., Napolioni V., Klepstad P., Belfer I., Molinaro V., Garbin G., Ranzani G.N., Alberio G., Normanno M., Lovisari F., Somaini M., Govoni S., Mura E., Bugada D., Niebel T., Zorzetto M., De Gregori S., Molinaro M., Fanelli G., Allegri M. 2016; Human Genetic Variability Contributes to Postoperative Morphine Consumption. *The Journal of Pain* **17** (5) pp. 628-636.

De Gregori, M., Garbin, G., De Gregori, S., Minella, C.E., Bugada, D., Lisa, A., Govoni, S., Regazzi, M., Allegri, M., Ranzani, G.N., 2013 Genetic Variability at COMT but Not at *OPRM1* and UGT2B7 Loci Modulates Morphine Analgesic Response in Acute Postoperative Pain. *European Journal of Clinical Pharmacology,* **69** (9) pp. 1651-1658.

de Luis, D., Aller, R., Izaola, O., Conde, R., de la Fuente, B., Gonzalez Sagrado, M., 2013a Genetic Variation in the Endocannabinoid Degrading Enzyme Fatty Acid Amide Hydrolase (FAAH) and their Influence on Weight Loss and Insulin Resistance Under a High Monounsaturated Fat Hypocaloric Diet. *Journal of Diabetes & its Complications,* **27** (3) pp. 235-239.

de Luis, D.A., Izaola, O., Aller, R., de La Fuente, B., Pacheco, D., 2013b Effects of C358A Polymorphism of the Endocannabinoid Degrading Enzyme Fatty Acid Amide Hydrolase (FAAH) on Weight Loss, Adipocytokines Levels, and Insulin Resistance After a High Polyunsaturated Fat Diet in Obese Patients. *Journal of Endocrinological Investigation,* **36** (11) pp. 965-969.

de Luis, D.A., Aller, R., Izaola, O., Conde, R., Sagrado, M.G., Primo, D., Castro, M.J., 2012 Relationship among Metabolic Syndrome, C358A Polymorphism of the Endocannabinoid Degrading Enzyme Fatty Acid Amide Hydrolase (FAAH) and Insulin Resistance. *Journal of Diabetes & its Complications,* **26** (4) pp. 328-332.

de Luis, D.A., Gonzalez Sagrado, M., Aller, R., Izaola, O., Conde, R., 2010 Relation of C358A Polymorphism of the Endocannabinoid Degrading Enzyme Fatty Acid Amide Hydrolase (FAAH) with Obesity and Insulin Resistance. *Nutricion Hospitalaria,* **25** (6) pp. 993-998.

Diatchenko, L., Slade, G.D., Nackley, A.G., Bhalang, K., Sigurdsson, A., Belfer, I., Goldman, D., Xu, K., Shabalina S.A., Shagin, D., Max, M.B., Makarov, S.S., Maixner, W., 2005 Genetic Basis for Individual Variations in Pain Perception and the Development of a Chronic Pain Condition, *Human Molecular Genetics,* **14** (1), pp. 135-143.

Dincheva, I., Drysdale, A.T., Hartley, C.A., Johnson, D.C., Jing, D., King, E.C., Ra, S., Gray, J.M., Yang, R., DeGruccio, A.M., Huang, C., Cravatt, B.F., Glatt, C.E., Hill, M.N., Casey, B.J., Lee, F.S., 2015 FAAH Genetic Variation Enhances Fronto-Amygdala Function in Mouse and Human. *Nature Communications,* **6** pp. 6395.

Ding, S., Chen, B., Zheng, Y., Lu, Q., Liu, L., Zhuge, Q., 2013 Association Study of *OPRM1* Polymorphisms with Schizophrenia in Han Chinese Population. *BMC Psychiatry,* **13** pp. 107.

Dlugos, A.M., Hamidovic, A., Hodgkinson, C.A., Goldman, D., Palmer, A.A., de Wit, H., 2010 More Aroused, Less Fatigued: Fatty Acid Amide Hydrolase Gene Polymorphisms Influence Acute Response to Amphetamine. *Neuropsychopharmacology,* **35** (3) pp. 613-622.

Drenth, J.P.H., Waxman, S.G., 2007 Mutations in Sodium-Channel Gene SCN9A Cause a Spectrum of Human Genetic Pain Disorders, *The Journal of Clinical Investigation,* **117** (12) pp. 3603-3609.

Dubin, A.E., Patapoutian, A., 2010 Nociceptors: The Sensors of the Pain Pathway, *The Journal of Clinical Investigation,* **120** (11) pp. 3760-3772.

Eccleston, C., & Crombez, G., 1999 Pain demands attention: A cognitive-affective model of the interruptive function of pain. Psychological Bulletin, 12 5(3), 356-366.

Eiselt, R., Domanski, T.L., Zibat, A., Mueller, R., Presecan-Siedel, E., Hustert, E., Zanger, U.M., Brockmoller, J., Klenk, H.P., Meyer, U.A., Khan, K.K., He, Y.A., Halpert, J.R., Wojnowski, L., 2001 Identification and Functional Characterization of Eight CYP3A4 Protein Variants, *Pharmacogenetics,* **11** (5) pp. 447-458.

Elens, L., Nieuweboer, A. Clarke, S.J., Charles, K.A., de Graan A-J., Haufroid, V., Mathijssen R.H.J., van Schaik, R.H.N., 2013 CYP3A4 Intron 6 C>T SNP (CYP3A4*22) Encodes Lower CYP3A4 Activity in Cancer Patients, as Measured with Probes Midazolam and Erythromycin., *Pharmacogenomics,* **14** (2), pp. 137-149.

Elens, L., Becker, M.L., Haufroid, V., Hofman, A., Visser, L.E., Uitterlinden, A.G., Stricker, B.C., van Schaik, R.H., 2011 Novel CYP3A4 Intron 6 Single Nucleotide Polymorphism is Associated with Simvastatin-Mediated Cholesterol Reduction in the Rotterdam Study, *Pharmacogenetics and Genomics,* **21** (12) pp. 861-866.

Elens, L., van Schaik, R.H., Panin, N., de Meyer, M., Wallemacq, P., Lison, D., Mourad, M., Haufroid, V., 2011 Effect of a New Functional CYP3A4 Polymorphism on Calcineurin Inhibitors' Dose Requirements and Trough Blood Levels in Stable Renal Transplant Patients, *Pharmacogenomics,* **12** (10) pp. 1383-1396.

Engeli, S., 2008 Dysregulation of the Endocannabinoid System in Obesity, *Journal of Neuroendocrinology,* **20** Suppl 1, pp. 110-115.

Fillingim, R.B., Kaplan, L., Staud, R., Ness, T.J., Glover, T.L., Campbell, C.M., Mogil, J.S., Wallace, M.R., 2005 The A118G Single Nucleotide Polymorphism of the Mu-Opioid Receptor Gene (*OPRM1*) is Associated with Pressure Pain Sensitivity in Humans. *Journal of Pain,* **6** (3) pp. 159-167.

Fishman S.M., 2012 Bonica's Management of Pain: Pharmacodynamic effects of opioids. *Lippincott Williams & Wilkins* pp. 1176

Fjell, A.M., Walhovd, K.B., Fennema-Notestine, C., McEvoy, L.K., Hagler, D.J., Holland, D., Brewer, J.B., Dale, A.M., 2009 One-Year Brain Atrophy Evident in Healthy Aging, *The Journal of Neuroscience,* **29** (48) pp. 15223-15231

Flanagan, J.M., Gerber, A.L., Cadet, J.L., Beutler, E., Sipe, J.C., 2006 The Fatty Acid Amide Hydrolase 385 A/A (P129T) Variant: Haplotype Analysis of an Ancient Missense Mutation and Validation of Risk for Drug Addiction. *Human Genetics,* **120** (4) pp. 581-588.

Frances, F., Portoles, O., Castello, A., Costa, J.A., Verdu, F., 2015 Association between Opioid Receptor Mu 1 (*OPRM1*) Gene Polymorphisms and Tobacco and Alcohol Consumption in a Spanish Population. *Bosnian Journal of Basic Medical Sciences,* **15** (2) pp. 31-36.

Freund, T.F., Katona, I., Piomelli, D., 2003 Role of Endogenous Cannabinoids in Synaptic Signaling, *Physiological Reviews,* **83** (3), pp. 1017-1066.

Fukuda, K., Hayashida, M., Ikeda, K., Koukita, Y., Ichinohe, T., Kaneko, Y., 2010 Diversity of Opioid Requirements for Postoperative Pain Control Following Oral Surgery--is it Affected by Polymorphism of the u-Opioid Receptor?., *Anesthesia Progress,* **57** (4), pp. 145-149.

Fukuda, K., Hayashida, M., Ide, S., Saita, N., Kokita, Y., Kasai, S., Nishizawa, D., Ogai, Y., Hasegawa, J., Nagashima, M., Tagami, M., Komatsu, H., Sora, I., Koga, H., Kaneko, Y., Ikeda, K., 2009 Association between *OPRM1* Gene Polymorphisms and Fentanyl Sensitivity in Patients Undergoing Painful Cosmetic Surgery. *Pain,* **147** (1-3) pp. 194-201.

Fukushima-Uesaka, H., Saito, Y., Watanabe, H., Shiseki, K., Saeki, M., Nakamura, T., Kurose, K., Sai, K., Komamura, K., Ueno, K., Kamakura, S., Kitakaze, M., Hanai, S., Nakajima, T., Matsumoto, K., Saito, H., Goto, Y., Kimura, H., Katoh, M., Sugai, K., Minami, N., Shirao, K., Tamura, T., Yamamoto, N., Minami, H., Ohtsu, A., Yoshida, T., Saijo, N., Kitamura, Y., Kamatani, N., Ozawa, S., Sawada, J., 2004 Haplotypes of CYP3A4 and their Close Linkage with CYP3A5 Haplotypes in a Japanese Population, *Human Mutation,* **23** (1) pp. 100.

Göker, M., Devoogdt, N., Van de Putte, G., Schobbens, J.C., Vlasselaer, J., Van de Broecke, R., de Jonge E.T.M., 2013 Systematic Review of Breast Cancer Related Lymphoedema: Making a Balanced Decision to Perform an Axillary Clearance, F*acts, Views & Vision in ObGyn,* **5** (2), pp. 106-115.

Gaedigk, A., Simon, S.D., Pearce, R.E., Bradford, L.D., Kennedy, M.J., Leeder, J.S., 2008 The CYP2D6 Activity Score: Translating Genotype Information into a Qualitative Measure of Phenotype, *Clinical Pharmacology & Therapeutics,* **83** (2), pp. 234-242.

Geha, P.Y., Baliki, M.N., Harden, R.N., Bauer, W.R., Parish, T.B., Apkarian, A.V., 2008 The Brain in Chronic CRPS Pain: Abnormal Gray-White Matter Interactions in Emotional and Autonomic Regions, *Neuron,* **60** (4), pp. 570-581.

Gerbershagen, H.J., Aduckathil, S., van Wijck, A.J.M., Peelen, L.M., Kalkman, C.J., Meissner, W., 2013 Pain Intensity on the First Day After Surgery: A Prospective Cohort Study Comparing 179 Surgical Procedures, *Anesthesiology,* **118** (4), pp. 934-944.

Giordano, J., 2005 The Neurobiology of Nociceptive and Anti-Nociceptive Systems., *Pain Physician,* **8** (3), pp. 277-290.

Greenspan, J.D., Lee, R.R., Lenz, F.A., 1999 Pain Sensitivity Alterations as a Function of Lesion Location in the Parasylvian Cortex., *Pain,* **81** (3), pp. 273-282.

Guasti, L., Zanotta, D., Diolisi,A., Carganico, D., Simoni, C., Gaudio, G., Grandi, A.M., Venco, A., 2002 Changes in Pain Perception during Treatment with Angiotensin Converting Enzyme-Inhibitors and Angiotensin II Type 1 Receptor Blockade, *Journal of Hypertension,* **20** (3), pp. 485-491.

Guzmán M., 2013 Cannabinods: potential anticancer agents, *Nature Rexiews Cancer,* **3**, p. 745-755

Haerian, B.S., Haerian, M.S., 2013 *OPRM1* rs1799971 Polymorphism and Opioid Dependence: Evidence from a Meta-Analysis, *Pharmacogenomics,* **14** (7) pp. 813-824.

Hagelberg, N.M., Nieminen, T.H., Saari, T.I., Neuvonen, M., Neuvonen, P.J., Laine, K., Olkkola, K., 2009 Voriconazole Drastically Increases Exposure to Oral Oxycodone., *European Journal of Clinical Pharmacology,* **65** (3), pp. 263-271.

Hariri, A.R., Gorka, A., Hyde, L.W., Kimak, M., Halder, I., Ducci, F., Ferrell, R.E., Goldman, D., Manuck, S.B., 2009 Divergent Effects of Genetic Variation in Endocannabinoid Signaling on Human Threat- and Reward-Related Brain Function. *Biological Psychiatry,* **66** (1) pp. 9-16.

Hassan, H.E., Myers, A.L., Lee, I.J., Coop, A., Eddington, N.D., 2007 Oxycodone Induces Overexpression of P-glycoprotein (ABCB1) and Affects Paclitaxel's Tissue Distribution in Sprague Dawley Rats, *Journal of Pharmaceutical Sciences,* **96** (9) pp. 2494-2506.

Hastie, B.A., Riley, J.L. 3rd, Kaplan, L., Herrera, D.G., Campbell, C.M., Virtusio, K., Mogil, J.S., Wallace, M.R., Fillingim, R.B., 2012 Ethnicity Interacts with the *OPRM1* Gene in Experimental Pain Sensitivity., *Pain,* **153** (8), pp. 1610-1619.

Hawker, G.A., Samra, M., Kendzerska, T., French, M., 2011 Measures of Adult Pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP), *Arthritis Care & Research,* **63** (11), pp. 240-252.

Haughey, H.M., Marshall, E., Schacht, J.P., Louis, A., Hutchison, K.E., 2008 Marijuana Withdrawal and Craving: Influence of the Cannabinoid Receptor 1 (CNR1) and Fatty Acid Amide Hydrolase (FAAH) Genes. *Addiction,* **103** (10) pp. 1678-1686.

Hayashida, M., Nagashima, M., Satoh, Y., Katoh, R., Tagami, M., Ide, S., Kasai, S., Nishizawa, D., Ogai, Y., Hasegawa, J., Komatsu, H., Sora, I., Fukuda, K. ,Koga, H., Hanaoka, K., Ikeda, K., 2008 Analgesic Requirements After Major Abdominal Surgery are Associated with *OPRM1* Gene Polymorphism Genotype and Haplotype. *Pharmacogenomics,* **9** (11) pp. 1605-1616.

Heiskanen, T., Olkkola, K.T., Kalso, E., 1998 Effects of Blocking CYP2D6 on the Pharmacokinetics and Pharmacodynamics of Oxycodone. *Clinical Pharmacology & Therapeutics,* **64** (6) pp. 603-611.

Henker, R.A., Lewis, A., Dai, F., Lariviere, W.R., Meng, L., Gruen, G.S., Sereika, S.M., Pape, H., Tarkin, I.S., Gowda, I., Conley, Y.P., 2013 The Associations between OPRM 1 and COMT Genotypes and Postoperative Pain, Opioid use, and Opioid-Induced Sedation, *Biological Research for Nursing,* **15** (3) pp. 309-317.

Henry, B., Graves, M.J., Pekala, J.R., Sanna, B., Hsieh, W.C., Tubbs, R.S., Walocha, J.A., Tomaszewski, K.A., 2017 Origin, Branching, and Communications of the Intercostobrachial Nerve: a Meta-Analysis with Implications for Mastectomy and Axillary Lymph Node Dissection in Breast Cancer, *Cureus* **9** (3), e1101

Hoenicka, J., Ponce, G., Jimenez-Arriero, M.A., Ampuero, I., Rodriguez-Jimenez, R., Rubio, G., Aragues, M., Ramos, J.A., Palomo, T., 2007 Association in Alcoholic Patients between Psychopathic Traits and the Additive Effect of Allelic Forms of the CNR1 and FAAH Endocannabinoid Genes, and the 3' Region of the DRD2 Gene. *Neurotoxicity Research,* **11** (1) pp. 51-60.

Hsieh, J.C., Ståhle-Bäckdahl, M., Hagermark, Ö., Stone-Elander, S., Rosenquist, G., Inqvar, M., 1996 Traumatic Nociceptive Pain Activates the Hypothalamus and the Periaqueductal Gray: A Positron Emission Tomography Study., *Pain,* **64** (2), pp. 303-314.

Huang, P., Chen, C., Mague, S.D., Blendy, J.A., Liu-Chen, L.Y., 2012 A Common Single Nucleotide Polymorphism A118G of the Mu Opioid Receptor Alters its N-Glycosylation and Protein Stability., *Biochemical Journal,* **441** (1), pp. 379-386.

Ip, H.Y.V., Abrishami A, Peng, P.W.H., Wong, J., Chung, F., 2009 Predictors of Postoperative Pain and Analgesic Consumption: A Qualitative Systematic Review, *Anesthesiology,* **111** (3), pp. 657-677.

Iwasaki, S., Ishiguro, H., Higuchi, S., Onaivi, E.S., Arinami, T., 2007 Association Study between Alcoholism and Endocannabinoid Metabolic Enzyme Genes Encoding Fatty Acid Amide Hydrolase and Monoglyceride Lipase in a Japanese Population. *Psychiatric Genetics,* **17** (4) pp. 215-220.

Jacobson, P.A., Oetting, W.S., Brearley, A.M., Leduc, R., Guan, W., Schladt, D., Matas, A.J., Lamba, V., Julian, B.A., Mannon, R.B., Israni, A., DeKAF Investigators, 2011 Novel Polymorphisms Associated with Tacrolimus Trough Concentrations: Results from a Multicenter Kidney Transplant Consortium, *Transplantation,* **91** (3) pp. 300-308.

Janicki, P.K., Schuler, G., Francis, D., Bohr, A., Gordin, V., Jarzembowski, T., Ruiz-Velasco, V., Mets, B., 2006 A Genetic Association Study of the Functional A118G Polymorphism of the Human Mu-Opioid Receptor Gene in Patients with Acute and Chronic Pain. *Anesthesia & Analgesia,* **103** (4) pp. 1011-1017.

Jensen, D.P., Andreasen, C.H., Andersen, M.K., Hansen, L., Eiberg, H., Borch-Johnsen, K., Jorgensen, T., Hansen, T., Pedersen, O., 2007 The Functional Pro129Thr Variant of the FAAH Gene is Not Associated with various Fat Accumulation Phenotypes in a Population-Based Cohort of 5,801

Whites. *Journal of Molecular Medicine,* **85** (5) pp. 445-449.

Jiang, Y., Nie, Y., Li, Y., Zhang, L., 2014 Association of Cannabinoid Type 1 Receptor and Fatty Acid Amide Hydrolase Genetic Polymorphisms in Chinese Patients with Irritable Bowel Syndrome. *Journal of Gastroenterology & Hepatology,* **29** (6) pp. 1186-1191.

Jones, A., Spindler, H., Jørgensen, M. M., & Zachariae, R., (2002). The effect of situation–evoked anxiety and gender on pain report using the cold pressor test. *Scandinavian Journal of Psychology*, **43** (4), 307-313.

Kalra, J., Chaturvedi, A., Kalra, S., Chatuvedi, H., Chasmana, D.C., 2008 Modulation of Pain Perception by Ramipril and Losartan in Human Volunteers, *Indian Journal of Physiology and Pharmacology,* **52** (1), pp. 91-96.

Kalso, E., Vainio, A., 1990 Morphine and Oxycodone Hydrochloride in the Management of Cancer Pain, *Clinical Pharmacology & Therapeutics,* **47** (5) pp. 639-646.

Kang, Y.S., Park, S.Y., Yim, C.H., Kwak, H.S., Galendrarao, P., Krishnamoorthy, N., Yun, S-C., Lee, K.W., Han, K.O., 2009 The CYP3A4*18 Genotype in the Cytochrome P450 3A4 Gene, a Rapid Metabolizer of Sex Steroids, is Associated with Low Bone Mineral Density, *Clinical Pharmacology & Therapeutics,* **85** (3), pp. 312-318.

Kaunisto M.A., Jokela, R., Tallgren, M., Kambur, O., Tikkanen, E., Tasmuth, T., Sipilä, R, Palotie, A., Estlander, A-M., Leidenius, M., Ripatti, S., Kalso, E., 2013 Pain in 1,000 Women Treated for Breast Cancer: A Prospective Study of Pain Sensitivity and Postoperative Pain., *Anesthesiology,* **119** (6), pp. 1410-1421.

Khan, A., Chakravorty, A., Gui, G.P.H., 2012 In Vivo Study of the Surgical Anatomy of the Axilla., *British Journal of Surgery,* **99** (6), pp. 871-877.

Keogh, E., & Cochrane, M. (2002). Anxiety sensitivity, cognitive biases, and the experience of pain. The Journal of Pain: Official Journal of the American Pain Society, 3(4), 320-329. doi: S1526590002000202 [pii]

Kim, H., Mittal, D.P., Iadarola,M.J., Dionne, R.A., 2006 Genetic Predictors for Acute Experimental Cold and Heat Pain Sensitivity in Humans, *Journal of Medical Genetics,* **43** (8), pp. e40

Kim, H., Clark, D., Dionne, R.A., 2009a Genetic Contributions to Clinical Pain and Analgesia: Avoiding Pitfalls in Genetic Research, *Journal of Pain,* **10** (7), pp. 663-693.

Kim, H., Ramsay, E., Lee, H., Wahl, S., Dionne, R.A., 2009b Genome-Wide Association Study of Acute Post-Surgical Pain in Humans., *Pharmacogenomics,* **10** (2), pp. 171-179.

Kim, H., Neubert, J.K., Rowan, J.S., Brahim, J.S., Iadarola, M.J., Dionne R.A., 2004 Comparison of Experimental and Acute Clinical Pain Responses in Humans as Pain Phenotypes. *The Journal of Pain* **5** (7), 377-84.

Kim, K.M., Kim, H.S., Lim, S.H. Cheong, S.H. Choi, E.J., Kang, H., Choi, H.R., Jeon, J.W., Yon, J.H., Oh, M., Shin, J.G., 2013 Effects of Genetic Polymorphisms of *OPRM1*, ABCB1, CYP3A4/5 on Postoperative Fentanyl Consumption in Korean Gynecologic Patients., *International Journal of Clinical Pharmacology & Therapeutics,* **51** (5), pp. 383-392.

Knowlton, W.M., Bifolck-Fisher, A., Bautista, D.M., McKemy, D.D., 2010 TRPM8, but Not TRPA1, is Required for Neural and Behavioral Responses to Acute Noxious Cold Temperatures and Cold-Mimetics in Vivo., *Pain,* **150** (2), pp. 340-350.

Ko M.L., King M.A., Gordon T.L., Crisp T., 1997 The Effects of Aging on Spinal Neurochemistry in the Rat. *Brain Research Bulletin* **42** (2) 95-98

Kokki, M., Välitalo, P., Kuusisto, M., Ranta, V.P., Raatikainen, K., Hautajärvi, H., Kokki, H., 2014 Central Nervous System Penetration of Oxycodone After Intravenous and Epidural Administration, *BJA: British Journal of Anaesthesia,* **112** (1), pp. 133-140.

Kokki, M., Broms, S., Eskelinen, M., Neuvonen, P.J., Halonen, T., Kokki, H., 2012a The Analgesic Concentration of Oxycodone with Co-Administration of Paracetamol? A Dose-Finding Study in Adult Patients Undergoing Laparoscopic Cholecystectomy, *Basic & Clinical Pharmacology & Toxicology,* **111** (6), pp. 391-395.

Kokki, M., Broms, S., Eskelinen, M., Rasanen, I., Ojanperä, I., Kokki, H., 2012b Analgesic Concentrations of Oxycodone? A Prospective Clinical PK/PD Study in Patients with Laparoscopic Cholecystectomy, *Basic & Clinical Pharmacology & Toxicology,* **110** (5), pp. 469-475.

Kolesnikov, Y., Gabovits, B., Levin, A., Voiko, E., Veske, A., 2011 Combined Catechol-O-Methyltransferase and Mu-Opioid Receptor Gene Polymorphisms Affect Morphine Postoperative Analgesia and Central Side Effects. *Anesthesia & Analgesia,* **112** (2) pp. 448-453.

Kuehl, P., Zhang, J., Lin, Y., Lamba, J., Assem, M., Schuetz, J., Watkins, P.B., Daly, A., Wrighton, S.A., Hall, S.D., Maurel, P., Relling, M., Brimer, C., Yasude, K., Venkataramanan, R., Strom, S., Thummel, K., Boguski, M.S., Schuetz, E., 2001 Sequence Diversity in CYP3A Promoters and Characterization of the Genetic Basis of Polymorphic CYP3A5 Expression, *Nature Genetics,* **27** (4), pp. 383-391.

Kumar, D., Chakraborty, J., Das, S., 2012 Epistatic Effects between Variants of Kappa-Opioid Receptor Gene and A118G of Mu-Opioid Receptor Gene Increase Susceptibility to Addiction in Indian Population. *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* **36** (2) pp. 225-230.

Lalovic, B., Kharasch, E., Hoffer, C., Risler, L., Liu-Chen, L.Y., Shen, D.D., 2006. Pharmacokinetics and Pharmacodynamics of Oral Oxycodone in Healthy Human Subjects: Role of Circulating Active Metabolites, *Clinical Pharmacology & Therapeutics,* **79** (5), pp. 461-479.

Lamba, J.K., Lin, Y.S., Thummel, K., Daly, A., Watkins, P.B., Strom, S., Zhang, J., Schuetz, E.G. 2002 Common Allelic Variants of Cytochrome P4503A4 and their Prevalence in Different Populations, *Pharmacogenetics,* **12** (2) pp. 121-132.

Lautenbacher, S., Huber, C., Kunz, M., Parthum, A., Weber, P.G., Griessinger, N., Sittl, R., 2009 Hypervigilance as Predictor of Postoperative Acute Pain: Its Predictive Potency Compared with Experimental Pain Sensitivity, Cortisol Reactivity, and Affective State., *Clinical Journal of Pain,* **25** (2), pp. 92-100.

Le Bars D., Dickenson A.H., Besson J., 1979; Diffuse Noxious Inhibitory Controls (DNIC). I. Effects on Dorsal Horn Convergent Neurones in the Rat. *PAIN* **6** 283-304.

Lee, M.G., Kim, H.J., Lee, K.H., Choi, Y.S., 2016 The Influence of Genotype Polymorphism on Morphine Analgesic Effect for Postoperative Pain in Children, *The Korean Journal of Pain,* **29** (1) pp. 34-39.

Lee, M.R., Gallen, C.L., Zhang, X., Hodgkinson, C.A., Goldman, D., Stein, E.A., Barr, C.S., 2011 Functional Polymorphism of the Mu-Opioid Receptor Gene (*OPRM1*) Influences Reinforcement Learning in Humans. *PLoS ONE [Electronic Resource],* **6** (9) pp. e24203.

Lemberg K.K., Heiskanen T.E., Kontinen V.K., Kalso E.A., 2009 Pharmacology of Oxycodone: Does it Explain Why Oxycodone has Become a Bestselling Strong Opioid? *Scandinavian Journal of Pain* **1** (Supplement 1) S18-23.

Lemberg, K, Kontinen, V., Siiskonen, A., Viljakka, K, Yli-Kauhaluoma, J., Korpi, E., Kalso, E., 2006 Antinociception by Spinal and Systemic Oxycodone: Why does the Route make a Difference?in Vitroand in VivoStudies in Rats, *Anesthesiology,* **105** (4) pp. 801-812.

Leow, K.P., Cramond, T., Smith, M.T., 1995 Pharmacokinetics and Pharmacodynamics of Oxycodone when Given Intravenously and Rectally to Adult Patients with Cancer Pain., *Anesthesia & Analgesia,* **80** (2), pp. 296-302.

Lewin, R., Göransson, M., Elander, A., Thorarinsson, A., Lundberg, J., Lidén, M., 2014 Risk Factors for Complications After Breast Reduction Surgery, *Journal of Plastic Surgery & Hand Surgery,* **48** (1), pp. 10-14.

Li, G.L., Winter, H., Arends, R., Jay, G.W., Le, V., Young, T., Huggins, J.P., 2012 Assessment of the Pharmacology and Tolerability of PF-04457845, an Irreversible Inhibitor of Fatty Acid Amide Hydrolase-1, in Healthy Subjects, *British Journal of Clinical Pharmacology,* **73** (5), pp. 706-716.

Liao, Q., Chen, D.J., Zhang, F., Li, L., Hu, R., Tang, Y.Z., Ou-Yang, W., Huang, D., 2013 Effect of CYP3A4*18B Polymorphisms and Interactions with *OPRM1* A118G on Postoperative Fentanyl Requirements in Patients Undergoing Radical Gastrectomy, *Molecular Medicine Reports,* **7** (3), pp. 901-908.

Lieb, W., Manning, A.K., Florez, J.C., Dupuis, J., Cupples, L.A., McAteer, J.B., Vasan, R.S., Hoffmann, U., O'Donnell, C.J., Meigs, J.B., Fox, C.S., 2009 Variants in the CNR1 and the FAAH Genes and Adiposity Traits in the Community. *Obesity,* **17** (4) pp. 755-760.

Lichtman, A.H., Leung, D., Shelton, C.C., Saghatelian, A., Hardouin, C., Boger, D.L., Cravatt, B.F., 2004 Reversible Inhibitors of Fatty Acid Amide Hydrolase that Promote Analgesia: Evidence for an Unprecedented Combination of Potency and Selectivity, *The Journal of Pharmacology and Experimental Therapeutics,* **311** (2), pp. 441-448.

Lin, Y.S., Dowling, A.L., Quigley, S.D., Farin, F.M., Zhang, J., Lamba, J., Schuetz, E.G., Thummel, K.E., 2002 Co-Regulation of CYP3A4 and CYP3A5 and Contribution to Hepatic and Intestinal Midazolam Metabolism, *Molecular Pharmacology,* **62** (1), pp. 162-172.

Liukas, A., Kuusniemi, K., Aantaa, R., Virolainen, P., Neuvonen, M., Neuvonen, P.J., Olkkola, K.T., 2011 Elimination of Intravenous Oxycodone in the Elderly: A Pharmacokinetic Study in Postoperative Orthopaedic Patients of Different Age Groups, *Drugs & Aging,* **28** (1), pp. 41-50.

Lu H.C., Mackie K., 2016 An Introduction to the Endogenous Cannabinoid System. *Biological Psychiatry,* **79** (7) 516-25.

Maihöfner C., Kaltenhäuser M., Neundörfer B., Lang E., 2002 Temporo-Spatial Analysis of Cortical Activation by Phasic Innocuous and Noxious Cold Stimuli – a Magnetoencephalographic Study. *Pain,* **100** (3) pp**.** 281-90.

Malhotra, B. K., Schoenhard, G.L., de Kater, A.W., Friedmann, N., 2015 The Pharmacokinetics of Oxycodone and its Metabolites Following Single Oral Doses of Remoxy(R), an Abuse-Deterrent Formulation of Extended-Release Oxycodone, in Patients with Hepatic Or Renal Impairment, *Journal of Opioid Management,* **11** (2), pp. 157-169.

Mallet, C., Dubray,C., Dualé, C., 2016 FAAH Inhibitors in the Limelight, but Regrettably, *International Journal of Clinical Pharmacology and Therapeutics,* **54** (7), pp. 498-501.

Manini, A.F., Jacobs, M.M., Vlahov, D., Hurd, Y.L., 2013 Opioid Receptor Polymorphism A118G Associated with Clinical Severity in a Drug Overdose Population. *Journal of Medical Toxicology: Official Journal of the American College of Medical Toxicology,* **9** (2) pp. 148-154.

Martinez, V., Fletcher, D., Bouhassira, D., Sessler, D., Chauvin, M., 2007 The Evolution of Primary Hyperalgesia in Orthopedic Surgery: Quantitative Sensory Testing and Clinical Evaluation before and After Total Knee Arthroplasty., *Anesthesia & Analgesia,* **105** (3), pp. 815-821.

Matic, M., van den Bosch, G.E., de Wildt, S.N., Tibboel, D., van Schaik, R.H., 2016 Genetic Variants Associated with Thermal Pain Sensitivity in a Paediatric Population, *Pain,* **157** 11, pp. 2476-2482.

Melroy-Greif, W.E., Wilhelmsen, K.C., Ehlers, C.L., 2016 Genetic Variation in FAAH is Associated with Cannabis use Disorders in a Young Adult Sample of Mexican Americans. *Drug & Alcohol Dependence,* **166** pp. 249-253.

Menon, S., Lea, R.A., Roy, B., Hanna, M., Wee, S., Haupt, L.M., Griffiths, L.R., 2012 The Human Mu-Opioid Receptor Gene Polymorphism (A118G) is Associated with Head Pain Severity in a Clinical Cohort of Female Migraine with Aura Patients. *Journal of Headache & Pain,* **13** (7) pp. 513-519.

Merzack R., 1975 The McGill Pain Questionnaire: major properties and scoring methods. *Pain* **1,** pp.275-299

Messerli, F., Ventura, H., Glade, L., Sundgaard-Riise, K., Dunn F., Frohlich E., 1983 Essential Hypertension in the Elderly: Haemodynamics, Intravascular Volume, Plasma Renin Activity, and Circulating Catecholamine Levels. *The Lancet* **322** (8357) 983-986.

Micale, V., Mazzola, C., Drago, F., 2007 Endocannabinoids and Neurodegenerative Diseases, *Pharmacological Research,* **56** (5), pp. 382-392.

Miguel, R., Kuhn, A.M., Shons, A.R., Dyches, P., Ebert, M.D., Peltz, E.S., Nguen, K., Cox, C.E., 2001 The Effect of Sentinel Node Selective Axillary Lymphadenectomy on the Incidence of Postmastectomy Pain Syndrome., *Cancer Control,* **8** (5), pp. 427-430.

Millan M.J., 1999 The Induction of Pain: An Integrative Review. *Progress in Neurobiology* **57** (1) pp. 1-164.

Mitjans, M., Gasto, C., Catalan, R., Fananas, L., Arias, B. 2012 Genetic Variability in the Endocannabinoid System and 12-Week Clinical Response to Citalopram Treatment: The Role of the CNR1, CNR2 and FAAH Genes. *Journal of Psychopharmacology,* **26** (10) pp. 1391-1398.

Monteleone, P., Milano, W., Petrella, C., Canestrelli, B., Maj, M., 2010 Endocannabinoid Pro129Thr FAAH Functional Polymorphism but Not 1359G/A CNR1 Polymorphism is Associated with Antipsychotic-Induced Weight Gain. *Journal of Clinical Psychopharmacology,* **30** (4) pp. 441-445.

Monteleone, P., Bifulco, M., Di Filippo, C., Gazzerro, P., Canestrelli, B., Monteleone, F., Proto, M.C., Di Genio, M., Grimaldi, C., Maj, M., 2009 Association of CNR1 and FAAH Endocannabinoid Gene Polymorphisms with Anorexia Nervosa and Bulimia Nervosa: Evidence for Synergistic Effects. *Genes, Brain, & Behavior,* **8** (7) pp. 728-732.

Monteleone, P., Tortorella, A., Martiadis, V., Di Filippo, C., Canestrelli, B., Maj, M., 2008 The cDNA 385C to A Missense Polymorphism of the Endocannabinoid Degrading Enzyme Fatty Acid Amide Hydrolase (FAAH) is Associated with overweight/obesity but Not with Binge Eating Disorder in overweight/obese Women. *Psychoneuroendocrinology,* **33** (4) pp. 546-550.

Moreira F.A., Kaiser N., Monory K., Lutz B., 2008; Reduced Anxiety-Like Behaviour Induced by Genetic and Pharmacological Inhibition of the Endocannabinoid-Degrading Enzyme Fatty Acid Amide Hydrolase (FAAH) is Mediated by CB1 Receptors. *Neuropharmacology* **54** 141-150.

Morin, C., Bushnell, M.C., 1998 Temporal and Qualitative Properties of Cold Pain and Heat Pain: A Psychophysical Study, *Pain,* **74** (1), pp. 67-73.

Morita, Y., Ujike, H., Tanaka, Y., Uchida, N., Nomura, A., Ohtani, K., Kishimoto, M., Morio, A., Imamura, T., Sakai, A., Inada, T., Harano, M., Komiyama, T., Yamada, M., Sekine, Y., Iwata, N., Iyo, M., Sora, I., Ozaki, N., Kuroda, S., 2005 A Nonsynonymous Polymorphism in the Human Fatty Acid Amide Hydrolase Gene did Not Associate with either Methamphetamine Dependence or Schizophrenia. *Neuroscience Letters,* **376** (3) pp. 182-187.

Moss, P., Whitnell, J., Wright, A., 2016 Quantitative and Qualitative Responses to Topical Cold in Healthy Caucasians show Variance between Individuals but High Test-Retest Reliability., *PLoS ONE [Electronic Resource],* **11** (3), pp. e0151972.

Muller, T.D., Bronner, G., Wandolski, M., Carrie, J., Nguyen, T.T., Greene, B.H., Scherag, A., Grallert, H., Vogel, C.I., Scherag, S., Rief, W., Wichmann, H., Illig, T., Schafer, H., Hebebrand, J., Hinney, A., 2010 Mutation Screen and Association Studies for the Fatty Acid Amide Hydrolase (FAAH) Gene and Early Onset and Adult Obesity. *BMC Medical Genetics,* **11** pp. 2.

Murayama, N., Nakamura, T., Saeki, M., Soyama, A., Saito, Y., Sai, K., Ishida, S., Nakajima, O., Itoda, M., Ohno, Y., Ozawa, S., Sawada, J., 2002 CYP3A4

Gene Polymorphisms Influence Testosterone 6beta-Hydroxylation, *Drug Metabolism and Pharmacokinetics,* **17** (2) pp. 150-156.

Myles, P.S., Iacono, G.A., Hunt, J.O., Fletcher, H., Morris, J., Mcllroy, D., Fritschi, L., 2002 Risk of Respiratory Complications and Wound Infection in Patients Undergoing Ambulatory Surgery: Smokers Versus Nonsmokers., *Anesthesiology,* **97** (4), pp. 842-847.

Nakamura, A., Hasegawa, M., Minami, K., Kanbara, T., Tomii, T., Nishiyori, A., Narita, M., Suzuki, T., Kato, A., 2013 Differential Activation of the mu-Opioid Receptor by Oxycodone and Morphine in Pain-Related Brain Regions in a Bone Cancer Pain Model, *British Journal of Pharmacology,* **168** (2) pp. 375-388.

Nelson, M.R., Wegmann, D., Ehm, M.G., Kessner, D., St. Jean, P., Verzilli, C., Shen, J., Tang, Z., Bacanu, S., Fraser, D., Warren, L., Aponte, J., Zawistowski, M., Liu, X., Zhang, H., Zhang, Y., Li, J., Li, Y., Li, L., Woollard, P., Topp, S., Hall, M.D., Nangle, K., Wang, J., Abecasis, G., Cardon, L.R., ZÃ¶llner, S., Whittaker, J.C., Chissoe, S.L., Novembre, J., Mooser, V., 2012 An Abundance of Rare Functional Variants in 202 Drug Target Genes Sequenced in 14,002 People, *Science,* **337** (6090) pp. 100.

Neuvonen M, Neuvonen PJ., 2008 Determination of Oxycodone, Noroxycodone, Oxymorphone, and Noroxymorphone in Human Plasma by Liquid Chromatography-Electrospray-Tandem Mass Spectrometry., *Therapeutic Drug Monitoring,* **30** (3), pp. 333-340.

Nielsen C.S., Stubhaug A., Price, D.D., Vassend O.F., Czajkowski N., Harris J.R., 2008 Individual Differences in Pain Sensitivity: Genetic and Environmental Contributions. *Pain* **136** (1-2), pp-21-29

Nielsen, L.M., Olesen, A.E., Sato, H., Christrup, L.L., Drewes, A.M., 2016 Association between Gene Polymorphisms and Pain Sensitivity Assessed in a Multi-Modal Multi-Tissue Human Experimental Model - an Explorative Study, *Basic & Clinical Pharmacology & Toxicology,* **119** (4) pp. 360-366.

Nieminen, T.H., Hagelberg, N.M., Saari, T.I., Neuvonen, M., Laine, K., Neuvonen, P.J., Olkkola K.T., 2010a St Johns Wort Greatly Reduces the Concentrations of Oral Oxycodone. *European Journal of Pain,* **14** (8), pp. 854-9.

Nieminen, T.H., Hagelberg, N.M., Saari, T.I., Neuvonen, M., Neuvonen, P.J., Laine, K., Olkkola, K.T., 2010 Oxycodone Concentrations are Greatly Increased by the Concomitant use of Ritonavir Or lopinavir/ritonavir. *European Journal of Clinical Pharmacology,* **66** (10) pp. 977-985.

Nieminen, T.H., Hagelberg, N.M., Saari, T.I., Pertovaara, A., Neuvonen, M., Laine, K, Neuvonen, P.J., Olkkola, K.T., 2009 Rifampin Greatly Reduces the Plasma Concentrations of Intravenous and Oral Oxycodone., *Anesthesiology,* **110** (6), pp. 1371-1378.

Norbury, T.A., MacGregor, A.J., Urwin, J., Spector, T.D., McMahon, S.B., 2007 Heritability of Responses to Painful Stimuli in Women: A Classical Twin Study, *Brain,* **130** (11) pp. 3041-3049.

Nurmi, E.L., Spilman, S.L., Whelan, F., Scahill, L.L., Aman, M.G., McDougle, C.J., Arnold, L.E., Handen, B., Johnson, C., Sukhodolsky, D.G., Posey, D.J., Lecavalier, L., Stigler, K.A., Ritz, L., Tierney, E., Vitiello, B., McCracken, J.T., 2013 Moderation of Antipsychotic-Induced Weight Gain by Energy Balance Gene Variants in the RUPP Autism Network Risperidone Studies. *Transl Psychiatry Psychiatry,* **3** pp. e274.

Oertel, B.G., Doehring, A., Roskam, B., Kettner, M., Hackmann, N., Ferreirós, N., Schmidt, P.H., Lötsch, J., 2012 Genetic–epigenetic Interaction Modulates μ-Opioid Receptor Regulation, *Human Molecular Genetics,* **21** (21) pp. 4751-4760

Olkkola K.T., Kontinen V.K., Saari T.I., Kalso E.A., 2013 Does the Pharmacology of Oxycodone Justify its Increasing use as an Analgesic? *Trends in Pharmacological Sciences,* **34** (4), pp. 206-214.

Orliaguet, G., Hamza, J., Couloigner, V., Denoyelle, F., Loriot, M.A., Broly, F., Garabedian, E.N., 2015 A Case of Respiratory Depression in a Child with Ultrarapid CYP2D6 Metabolism After Tramadol, *Pediatrics,* **135** (3) pp. e753-5.

Ossipov, M.H., Morimura, K., Porreca, F., 2014 Descending Pain Modulation and Chronification of Pain, *Current Opinion in Supportive and Palliative Care,* **8** (2) pp. 143-151.

Palkar R., Lippoldt E.K., McKemy D.D., 2015 The Molecular and Cellular Basis of Thermosensation in Mammals. *Current Opinion in Neurobiology,* **34** pp. 14-9.

Papazoglou, D., Panagopoulos, I., Papanas, N., Gioka, T., Papadopoulos, T., Papathanasiou, P., Kaitozis, O., Papatheodorou, K., Maltezos, E., 2008 The Fatty Acid Amide Hydrolase (FAAH) Pro129Thr Polymorphism is Not Associated with Severe Obesity in Greek Subjects. *Hormone & Metabolic Research,* **40** (12) pp. 907-910.

Pecina, M., Martinez-Jauand, M., Hodgkinson, C., Stohler, C.S., Goldman, D., Zubieta, J.K., 2014 FAAH Selectively Influences Placebo Effects. *Molecular Psychiatry,* **19** (3) pp. 385-391.

Peier, A.M., Moqrich, A., Hergarden, A.C., Reeve, A.J., Andersson, D.A., Story, G.M., Earley, T.J., Dragoni, I., McIntyre, P., Bevan, S., Papapoutian, A., 2002 A TRP Channel that Senses Cold Stimuli and Menthol., *Cell,* **108** (5), pp. 705-715.

Peier, A.M., Reeve, A.J., Andersson, D.A., Moqrich, A., Earley, T.J., Hergarden, A.C., Story, G.M.,

Colley, S., Hogenesch, J.B., McIntyre, P., Bevan, S., Patapoutian, A., 2002 A Heat-Sensitive TRP Channel Expressed in Keratinocytes, *Science,* **296** (5575) pp. 2046.

Pereira, M. P., Pogatzki-Zahn, E., 2015 Gender Aspects in Postoperative Pain, *Current Opinion in Anaesthesiology,* **28** (5), pp. 546-558.

Pickar, D., Cohen, M.R., Naber, D., Cohen, R.M., 1982 Clinical Studies of the Endogenous Opioid System, *Biological Psychiatry,* **17** (11) pp. 1243-1276.

Pietarinen, P., Tornio, A., Niemi, M., 2016 High Frequency of CYP2D6 Ultrarapid Metabolizer Genotype in the Finnish Population., *Basic & Clinical Pharmacology & Toxicology,* **119** (3), pp. 291-296.

Piirainen, A., Kokki, H., Immonen, S., Eskelinen, M., Häkkinen, M.R., Hautajärvi, H., Kokki, M., 2015 A Dose-Finding Study of Dexketoprofen in Patients Undergoing Laparoscopic Cholecystectomy: A Randomized Clinical Trial on Effects on the Analgesic Concentration of Oxycodone., *Drugs in R & D,* **15** (4), pp. 319-328.

Pisanu, C., Congiu, D., Costa, M., Sestu, M., Chillotti, C., Ardau, R., Deiana, V., Manchia, M., Squassina, A., Del Zompo, M., 2013 No Association of Endocannabinoid Genes with Bipolar Disorder or Lithium Response in a Sardinian Sample. *Psychiatry Research,* **210** (3) pp. 887-890.

Ploghaus, A., Narain, C., Beckmann, C.F., Clare, S., Bantick, S., Wise, R., Matthews, P.M., Rawlins, J.N.P., Tracey, I., 2001 Exacerbation of Pain by Anxiety is Associated with Activity in a Hippocampal Network, *The Journal of Neuroscience,* **21** (24), pp. 9896-9903.

Proudnikov, D., Randesi, M., Levran, O., Crystal, H., Dorn, M., Ott, J., Ho, A., Kreek, M.J., 2012 Association of Polymorphisms of the Mu Opioid Receptor Gene with the Severity of HIV Infection and Response to HIV Treatment. *Journal of Infectious Diseases,* **205** (11) pp. 1745-1756.

Proudnikov, D., Kroslak, T., Sipe, J.C., Randesi, M., Li, D., Hamon, S., Ho, A., Ott, J., Kreek, M.J., 2010 Association of Polymorphisms of the Cannabinoid Receptor (CNR1) and Fatty Acid Amide Hydrolase (FAAH) Genes with Heroin Addiction: Impact of Long Repeats of CNR1. *Pharmacogenomics Journal,* **10** (3) pp. 232-242.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A. Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., Sham, P.C., 2007 PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses., *American Journal of Human Genetics,* **81**, pp. 559-575.

Pöyhiä, R., Seppälä, T., 1994 Liposolubility and Protein Binding of Oxycodone in Vitro., *Pharmacology & Toxicology,* **74** (1), pp. 23-27.

Ray, L.A., Hutchison, K.E., 2004 A Polymorphism of the μ-Opioid Receptor Gene (*OPRM1*) and Sensitivity to the Effects of Alcohol in Humans, *Alcoholism: Clinical and Experimental Research,* **28** (12) pp. 1789-1795.

Ren, Z.Y., Xu, X.Q., Bao, Y.P., He, J., Shi, L., Deng, J.H., Gao, X.J., Tang, H.L., Wang, Y.M., Lu, L., 2015 The Impact of Genetic Variation on Sensitivity to Opioid Analgesics in Patients with Postoperative Pain: A Systematic Review and Meta-Analysis, *Pain Physician,* **18** (2), pp. 131-152.

Rouvinen-Lagerstrom, N., Lahti, J., Alho, H., Kovanen, L., Aalto, M., Partonen, T., Silander, K., Sinclair, D., Raikkonen, K., Eriksson, J.G., Palotie, A., Koskinen, S., Saarikoski, S.T., 2013 Mu-Opioid Receptor Gene (*OPRM1*) Polymorphism A118G: Lack of Association in Finnish Populations with Alcohol Dependence or Alcohol Consumption. *Alcohol & Alcoholism,* **48** (5) pp. 519-525.

Saari, T.I., Ihmsen, H., Neuvonen, P.J., Olkkola, K.T., Schwilden, H., 2012 Oxycodone Clearance is Markedly Reduced with Advancing Age: A Population Pharmacokinetic Study., *British Journal of Anaesthesia,* **108** (3), pp. 491-498.

Sadhasivam, S., Zhang, X., Chidambaran, V., Mavi, J., Pilipenko, V., Mersha, T.B., Meller, J., Kaufman, K.M., Martin, L.J., McAuliffe, J., 2015 Novel Associations between FAAH Genetic Variants and Postoperative Central Opioid-Related Adverse Effects. *Pharmacogenomics Journal,* **15** (5) pp. 436-442.

Sander, T., Berlin, W., Gscheidel, N., Wendel, B., Janz, D., Hoehe, M.R., 2000 Genetic Variation of the Human Mu-Opioid Receptor and Susceptibility to Idiopathic Absence Epilepsy, *Epilepsy Research,* **39** (1) pp. 57-61.

Sangesland, A., Storen, C., Vaegter. H.B., 2017 Are Preoperative Experimental Pain Assessments Correlated with Clinical Pain Outcomes After Surgery? A Systematic Review, *Scandinavian Journal of Pain,* **15** pp. 44-52.

Sarzani, R., Bordicchia, M., Salvi, F., Cola, G., Franchi, E., Battistoni, I., Mancinelli, L., Giovagnoli, A., Dessi-Fulgheri, P., Rappelli, A., 2008 A Human Fatty Acid Amide Hydrolase (FAAH) Functional Gene Variant is Associated with Lower Blood Pressure in Young Males. *American Journal of Hypertension,* **21** (8) pp. 960-963.

Schacht, J.P., Selling, R.E., Hutchison, K.E., 2009 Intermediate Cannabis Dependence Phenotypes and the FAAH C385A Variant: An Exploratory Analysis. *Psychopharmacology,* **203** (3) pp. 511-517.

Schmahmann, J. D., Leifer. D., 1992 Parietal Pseudothalamic Pain Syndrome. Clinical Features and Anatomic Correlates., *Archives of Neurology,* **49** (10), pp. 1032-1037.

Schutz, M., Oertel, B.G., Heimann, D., Doehring, A., Walter, C., Dimova, V., Geisslinger, G., Lötsch, J., 2014 Consequences of a Human TRPA1 Genetic Variant on the Perception of Nociceptive and Olfactory Stimuli., *PLoS ONE [Electronic Resource],* **9** (4), pp. e95592.

Schwantes-An, T., Zhang, J., Chen, L., Hartz, S.M., Culverhouse, R.C., Chen, X., Coon, H., Frank, J., Kamens, H.M., Konte, B., Kovanen, L., Latvala, A., Legrand, L.N., Maher, B.S., Melroy, W.E., Nelson, E.C., Reid, M.W., Robinson, J.D., Shen, P., Yang, B., Andrews, J.A., Aveyard, P., Beltcheva, O., Brown, S.A., Cannon, D.S., Cichon, S., Corley, R.P., Dahmen, N., Degenhardt, L., Foroud, T., Gaebel, W., Giegling, I., Glatt, S.J., Grucza, R.A., Hardin, J., Hartmann, A.M., Heath, A.C., Herms, S., Hodgkinson, C.A., Hoffmann, P., Hops, H., Huizinga, D., Ising, M., Johnson, E.O., Johnstone, E., Kaneva, R.P., Kendler, K.S., Kiefer, F., Kranzler, H.R., Krauter, K.S., Levran, O., Lucae, S., Lynskey, M.T., Maier, W., Mann, K., Martin, N.G., Mattheisen, M., Montgomery, G.W., Muller-Myhsok, B., Murphy, M.F., Neale, M.C., Nikolov, M.A., Nishita, D., Nothen, M.M., Nurnberger, J., Partonen, T., Pergadia, M.L., Reynolds, M., Ridinger, M., Rose, R.J., Rouvinen-Lagerstrom, N., Scherbaum, N., Schmal, C., Soyka, M., Stallings, M.C., Steffens, M., Treutlein, J., Tsuang, M., Wall, T.L., Wodarz, N., Yuferov, V., Zill, P., Bergen, A.W., Chen, J., Cinciripini, P.M., Edenberg, H.J., Ehringer, M.A., Ferrell, R.E., Gelernter, J., Goldman, D., Hewitt, J.K., Hopfer, C.J., Iacono, W.G., Kaprio, J., Kreek, M.J., Kremensky, I.M., Madden, P.A.F., McGue, M., Munafo, M.R., Philibert, R.A., Rietschel, M., Roy, A., Rujescu, D., Saarikoski, S.T., Swan, G.E., Todorov, A.A., Vanyukov, M.M., Weiss, R.B., Bierut, L.J., Saccone, N.L., 2016 Association of the *OPRM1* Variant rs1799971 (A118G) with Non-Specific Liability to Substance Dependence in a Collaborative De Novo Meta-Analysis of European-Ancestry Cohorts. *Behavior Genetics,* **46** (2) pp. 151-169.

Sery, O., Prikryl, R., Castulik, L., St'astny, F., 2010 A118G Polymorphism of *OPRM1* Gene is Associated with Schizophrenia. *Journal of Molecular Neuroscience,* **41** (1) pp. 219-222.

Shi, Y, Weingarten, T.N., Mantilla, C.B., Hooten, W.M., Warner, D.O., 2010 Smoking and Pain Pathophysiology and Clinical Implications, *Anesthesiology,* **113** (4) pp. 977-992.

Sia, A.T., Lim, Y., Lim, E.C.P., Goh, R.W.C., Law, H.Y., Landau, R., Teo, Y.Y., Tan, E.C., 2008 A118G Single Nucleotide Polymorphism of Human Mu-Opioid Receptor Gene Influences Pain Perception and Patient-Controlled Intravenous Morphine Consumption After Intrathecal Morphine for Postcesarean Analgesia., *Anesthesiology,* **109** (3), pp. 520-526.

Sia, A.T., Lim, Y., Lim, E.C.P., Ocampo, C.E., Lim, W.Y., Cheong, P., Tan E.C., 2013 Influence of Mu-Opioid Receptor Variant on Morphine use and Self-Rated Pain Following Abdominal Hysterectomy, *The Journal of Pain,* **14** (10), pp. 1045-1052

Sipe, J.C., Waalen, J., Gerber, A., Beutler, E., 2005 Overweight and Obesity Associated with a Missense Polymorphism in Fatty Acid Amide Hydrolase (FAAH). *International Journal of Obesity,* **29** (7) pp. 755-759.

Sipe, J.C., Chiang, K., Gerber, A.L., Beutler, E., Cravatt, B.F., 2002 A Missense Mutation in Human Fatty Acid Amide Hydrolase Associated with Problem Drug use. *Proceedings of the National Academy of Sciences of the United States of America,* **99** (12) pp. 8394-8399.

Sipila, R.M., Haasio, L., Meretoja, T.J., Ripatti, S., Estlander, A., Kalso, E.A., 2017 Does Expecting More Pain make it More Intense? Factors Associated with the First Week Pain Trajectories After Breast Cancer Surgery. *Pain,* **158** (5) pp. 922-930.

Somogyi, A.A., Sia, A.T., Tan, E.C., Coller, J.K., Hutchinson, M.R., Barratt, D.T., 2016 Ethnicity-Dependent Influence of Innate Immune Genetic Markers on Morphine PCA Requirements and Adverse Effects in Postoperative Pain., *Pain,* **157** (11), pp. 2458-2466.

Sorensen LT, Hemmingsen U, Kallehave F, Willie-Jorgensen, P., Kjaegaard, J., Moller, L.N., Jorgensen, T., 2005 Risk Factors for Tissue and Wound Complications in Gastrointestinal Surgery., *Annals of Surgery,* **241** (4), pp. 654-658.

Spagnolo, P.A., Ramchandani, V.A., Schwandt, M.L., Kwako, L.E., George, D.T., Mayo, L.M., Hillard, C.J., Heilig, M., 2016 FAAH Gene Variation Moderates Stress Response and Symptom Severity in Patients with Posttraumatic Stress Disorder and Comorbid Alcohol Dependence. *Alcoholism: Clinical & Experimental Research,* **40** (11) pp. 2426-2434.

Spielberger CD GR, Lushane PR, Vagg PR, Jacobs AG., 1983 Manual for the State-Trait Anxiety Inventory (Form Y), *Consulting Psychologists Press, Inc*, Palo Alto,

Storr, M., Emmerdinger, D., Diegelmann, J., Yuce, B., Pfennig, S., Ochsenkuhn, T., Goke, B., Lohse, P., Brand, S., 2009 The Role of Fatty Acid Hydrolase Gene Variants in Inflammatory Bowel Disease. *Alimentary Pharmacology & Therapeutics,* **29** (5) pp. 542-551.

Sugino, S., Hayase, T., Higuchi, M., Saito, K., Moriya, H., Kumeta, Y., Kurosawa, N., Namiki, A., Janicki, P.K., 2014 Association of Mu-Opioid Receptor Gene (*OPRM1*) Haplotypes with Postoperative Nausea and Vomiting. *Experimental Brain Research,* **232** (8) pp. 2627-2635.

Tan, E. C., Lim, E.C., Teo, Y.Y., Lim, Y., Law, H.Y., Sia, A., 2009 Ethnicity and OPRM Variant Independently

Predict Pain Perception and Patient-Controlled Analgesia Usage for Post-Operative Pain., *Molecular Pain,* **5** (32), pp. 32.

Tan, P. C. S., Hassan, S.K., Mohamad, N.A.N., Gan, S.H., 2012 Cytochrome P450 3A4 Genetic Polymorphisms and Post-Operative Fentanyl Requirements., *Journal of Clinical Pharmacy & Therapeutics,* **37** (1), pp. 100-104.

Tegeder, I., Costigan, M., Griffin, RS., Abele, A., Belfer, I., Schmidt, H., Ehnert, C., Nejim, J., Marian C., Scholz, J., Wu, T., Allchorne, A., Diatchenko, L, Binshtok, A.M., Goldman, D., Adolph, J., Sama, S., Atlas, S.J., Carlezon, W.A., Parsegian, A., Lötsch, J., Fillingim, R.B., Maixner, W., Geisslinger, G., Max, M.B., Woolf, C.J., 2006 GTP Cyclohydrolase and Tetrahydrobiopterin Regulate Pain Sensitivity and Persistence., *Nature Medicine,* **12** (11), pp. 1269-1277.

Tejera, P., Meyer, N.J., Chen, F., Feng, R., Zhao, Y., O'Mahony, D.S., Li, L., Sheu, C., Zhai, R., Wang, Z., Su, L., Bajwa, E., Ahasic, A.M., Clardy, P.F., Gong, M.N., Frank, A.J., Lanken, P.N., Thompson, B.T., Christie, J.D., Wurfel, M.M., O'Keefe, G.E., Christiani, D.C., 2012 Distinct and Replicable Genetic Risk Factors for Acute Respiratory Distress Syndrome of Pulmonary or Extrapulmonary Origin. *Journal of Medical Genetics,* **49** (11) pp. 671-680.

Tennessen, J.A., Bigham, A.W., O'Connor, T.D., Fu, W., Kenny, E.E., Gravel, S., McGee, S., Do, R., Liu, X., Jun, G., Kang, H.M., Jordan, D., Leal, S.M., Gabriel, S., Rieder, M.J., Abecasis, G., Altshuler, D., Nickerson, D.A., Boerwinkle, E., Sunyaev, S., Bustamante, C.D., Bamshad, M.J., Akey, J.M., 2012 Evolution and Functional Impact of Rare Coding Variation from Deep Sequencing of Human Exomes, *Science,* **337** (6090) pp. 64.

The ENCODE, P.C., 2012 An Integrated Encyclopedia of DNA Elements in the Human Genome, *Nature,* **489** pp. 57.

Thomazeau, J., Rouquette, A., Martinez, V., Rabuel, C., Prince, N., Laplanche, J.L., Nizard, R., Bergmann, J.F., Perrot, S., Lloret-Linares, C., 2016 Acute Pain Factors Predictive of Post-Operative Pain and Opioid Requirement in Multimodal Analgesia Following Knee Replacement, *European Journal of Pain,* **20** (5) pp. 822-832.

Trang, T., Al-Hasani, R., Salvemini, D., Salter, M.W., Gutstein, H., Cahill, C.M., 2015 Pain and Poppies: The Good, the Bad, and the Ugly of Opioid Analgesics, *The Journal of Neuroscience,* **35** (41) pp. 13879-13888

Tyndale, R.F., Payne, J.I., Gerber, A.L., Sipe, J.C., 2007 The Fatty Acid Amide Hydrolase C385A (P129T) Missense Variant in Cannabis Users: Studies of Drug use and Dependence in Caucasians. *American Journal of Medical Genetics.Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics,* **144B** (5) pp. 660-666.

Quanto Power Calculation Software; version 1.2.4: University of Southern California. http://hydra.usc.edu/gxe/

Verdú E., Dolores, C., Vilches, J.J., Xavier, N., 2000 Influence of Aging on Peripheral Nerve Function and Regeneration, *Journal of the Peripheral Nervous System,* **5** (4) pp. 191-208

Verhagen, M., Kleinjan, M., Engels, R.C., 2012 A Systematic Review of the A118G (Asn40Asp) Variant of *OPRM1* in Relation to Smoking Initiation, Nicotine Dependence and Smoking Cessation, *Pharmacogenomics,* **13** (8) pp. 917-933.

Villesen, H.H., Foster, D.J.R., Upton, R.N., Somogyi, A.A., Martinez, A., Grant, C., 2006 Cerebral Kinetics of Oxycodone in Conscious Sheep, *Journal of Pharmaceutical Sciences,* **95** (8), pp. 1666-1676.

Viveros M.P., Marco E.M., File S.E., 2005; Endocannabinoid System and Stress and Anxiety Responses. *Pharmacology Biochemistry and Behavior* **81** 331-342.

Walter, C., Lötsch, J., 2009 Meta-Analysis of the Relevance of the *OPRM1* 118A>G Genetic Variant for Pain Treatment, *Pain,* **146** (3) pp. 270-275.

Wang, D., Guo, Y., Wrighton, S.A., Cooke, G.E., Sadee, W., 2011 Intronic Polymorphism in CYP3A4 Affects Hepatic Expression and Response to Statin Drugs., *Pharmacogenomics Journal,* **11** (4), pp. 274-286.

Wang, D., Sadee, W., 2016 CYP3A4 Intronic SNP rs35599367 (CYP3A4*22) Alters RNA Splicing., *Pharmacogenetics and Genomics,* **26** (1), pp. 40-43.

Wang, J., Jin, P., Wang, W., He, M., Zhang, Z., Liu, Y., 2015 Association of A118G Polymorphism in the Mu-Opioid Receptor Gene with Smoking Behaviors: A Meta-Analysis. *Journal of Toxicological Sciences,* **40** (6) pp. 711-718.

Watanabe, H., Vriens, J., Suh, S.H., Benham, C.D., Droogmans, G., Nilius, B., 2002 Heat-Evoked Activation of TRPV4 Channels in a HEK293 Cell Expression System and in Native Mouse Aorta Endothelial Cells, *The Journal of Biological Chemistry,* **277** (49) pp. 47044-47051.

Wei, L.X., Floreani, A., Variola, A., El Younis, C., Bergasa, N.V., 2008 A Study of the Mu Opioid Receptor Gene Polymorphism A118G in Patients with Primary Biliary Cirrhosis with and without Pruritus, *Acta Dermato-Venereologica,* **88** (4) pp. 323-326.

Willard, Huntington F., 2013 Chapter 1 - the Human Genome: A Window on Human Genetics, Biology, and Medicine, *Genomic and Personalized Medicine (Second Edition)*, Academic Press, pp. 4-27.

Williams, F.M.K., Scollen, S., Cao, D., Memari, Y., Hyde, C.L., Zhang, B., Sidders, B., Ziemek, D., Shi, Y., Harris, J., Harrow, I., Dougherty, B., Malarstig, A., McEwen, R., Stephens, J.C., Patel, K., Menni, C., Shin, S., Hodgkiss, D., Surdulescu, G., He, W., Jin, X., McMahon, S.B., Soranzo, N., John, S., Wang, J., Spector, T.D., 2012 Genes Contributing to Pain Sensitivity in the Normal Population: An Exome Sequencing Study, *PLOS Genetics,* **8** (12) pp. e1003095.

Willis, W. D., Westlund, K.N., 1997 Neuroanatomy of the Pain System and of the Pathways that Modulate Pain, *Journal of Clinical Neurophysiology. Neurophysiology of Pain,* **14** (1), pp. 2-31.

Wu, K., Chen, C., Moyzis, R.K., Greenberger, E., Yu, Z., 2016 Gender Interacts with Opioid Receptor Polymorphism A118G and Serotonin Receptor Polymorphism -1438 A/G on Speed-Dating Success. *Human Nature,* **27** (3) pp. 244-260.

Xu, J., Lu, Z., Xu, M., Rossi, G.C., Kest, B., Waxman, A.R., Pasternak, G.W., Pan, Y., 2014 Differential Expressions of the Alternatively Spliced Variant mRNAs of the μ Opioid Receptor Gene, OPRM1, in Brain Regions of Four Inbred Mouse Strains, *Plos One,* **9** (10) pp. e111267

Yang, Z., Yang, Z., Arheart, K.L., Morris, R., Zhang, Y., Rodriques, Y., Song, C., Gitlin, M.C., Candiotti, K.A., 2012 CYP2D6 Poor Metabolizer Genotype and Smoking Predict Severe Postoperative Pain in Female Patients on Arrival to the Recovery Room., *Pain Medicine,* **13** (4), pp. 604-609.

Yarnitsky, D., 2015 Role of Endogenous Pain Modulation in Chronic Pain Mechanisms and Treatment, *Pain,* **156** (Suppl 1) pp. S24-31.

Zhang, F., Liao, Q., Li, L.I., Wang, S., Hu, R., Tang, Y., Ouyang, W., 2013 The Correlation between Post-Operative Fentanyl Requirements and Î¼-Opioid Receptor Gene A118G Polymorphism in Patients Undergoing Radical Gastrectomy, *Experimental and Therapeutic Medicine,* **5** (4) pp. 1147-1152.

Zhang, W., Yuan, J.J., Kan, Q.C., Zhang, L.R., Chang, Y.Z., Wang, Z.Y., 2011 Study of the *OPRM1* A118G Genetic Polymorphism Associated with Postoperative Nausea and Vomiting Induced by Fentanyl Intravenous Analgesia, *Minerva Anestesiologica,* **77** (1), pp. 33-39.

Zhang, W., Chang, Y.Z., Kan, Q.C., Zhang, L.R., Lu, H., Chu, Q.J., Wang, Z.Y., Li, S., Zhang, J., 2010 Association of Human μ-Opioid Receptor Gene Polymorphism A118G with Fentanyl Analgesia Consumption in Chinese Gynaecological Patients, *Anaesthesia,* **65** (2), pp. 130-135.

Zhang, Y., Sonnenberg, G.E., Baye, T.M., Littrell, J., Gunnell, J., DeLaForest, A., MacKinney, E., Hillard, C.J., Kissebah, A.H., Olivier, M., Wilke, R.A., 2009 Obesity-Related Dyslipidemia Associated with FAAH, Independent of Insulin Response, in Multigenerational Families of Northern European Descent. *Pharmacogenomics,* **10** (12) pp. 1929-1939.

Zhang, Y., Wang, D., Johnson, A.D., Papp, A.C., Sadée, W., 2005 Allelic Expression Imbalance of Human Mu Opioid Receptor (OPRM1) Caused by Variant A118G, *Journal of Biological Chemistry,* **280** (38) pp. 32618-32624

Zheng, H., Schnabel, A., Yahiaouir, M., Meissner, W., Van Aken, H., Zahn, P., Pogatzki-Zahn, E., 2017 Age and Preoperative Pain are Major Confounders for Sex Differences in Postoperative Pain Outcome: A Prospective Database Analysis, *Plos One,* **12** (6) pp. e0178659.

Zhou, S.F., 2009a Polymorphism of Human Cytochrome P450 2D6 and its Clinical Significance: Part II, *Clinical Pharmacokinetics,* **48** (12), pp. 761-804.

Zhou, S.F., 2009b Polymorphism of Human Cytochrome P450 2D6 and its Clinical Significance: Part I, *Clinical Pharmacokinetics,* **48** (11), pp. 689-723.

Zwisler, S.T., Enggaard, T.P., Mikkelsen, S., Brosen, K., Sindrup, S.H., 2010 Impact of the CYP2D6 Genotype on Post-Operative Intravenous Oxycodone Analgesia, *Acta Anaesthesiologica Scandinavica,* **54** (2), pp. 232-240.

Zwisler, S.T., Enggaard, T.P., Verstuyft, C., Becquemont, L., Sindrup.S.H., Brosen, K., 2012 Lack of Association of *OPRM1* and ABCB1 Single-Nucleotide Polymorphisms to Oxycodone Response in Postoperative Pain, *Journal of Clinical Pharmacology,* **52** (2), pp. 234-242.

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